

9 July 1981

British universities on the brink

The threat of execution is said to help to clarify the mind. The British university system, which has woken up to the threat of trouble ahead only within the past year, will not even profit by knowing where it stands from the announcement last week of the allocations of funds to individual universities for the three years ahead (see page 99). The dilemma is constitutionally appropriate. The University Grants Committee has dealt with the universities as it should — self-governing institutions as they like to think they are, they have been told to manage their own affairs as best they can within the funds made available for the next few years. But some options the universities are individually and collectively denied. Because the grants committee has coupled its financial allocations with specific instructions about the numbers of domestic and European Community students individual universities may in future accommodate, it will not be possible for them to work their way out of trouble by being more productive. (If ever the universities have time, the legality of this requirement might usefully be tested in the courts.) And because the universities that have come well out of the past week's lottery are unlikely, self-governing as they are, to throw in their lot with the less fortunate, it is probable that half a dozen of the places most seriously affected will have to sink or swim on their own. Some of them, faced with budget cuts of 25 per cent or so (Aston in Birmingham and Salford for example) will be lucky if they do not sink, and will even now be asking whether that is the surreptitious intention of either the government or the grants committee.

For while the committee's announcement last week will certainly leave its mark on the British university system, there are still too many loose ends for comfort. What will in the end most affect the universities whose budgets are most drastically to be cut will be the lack of any formal mechanism for paying off redundant members of university staffs. Although it is agreed that the universities on which the cuts fall hardest will be able to adjust only by getting rid of tenured academics, no funds are available for providing them with compensation. And although Dr Edward Parkes, the chairman of the University Grants Committee, told a House of Commons select committee last year that some £200 million would be needed to bring about the impending adjustment, merely £20 million has been set aside for such purposes (out of the universities' collective subvention). But should not academics suffer the indignities and impoverishments that seem certain to afflict some three million other unemployed people in the United Kingdom before the year is out? This is what the enemies of the universities are asking. Some such calculation may account for the spleen with which the government has pursued the universities since its election more than two years ago. The trouble is that tenured academics most probably have rights at common law against universities that dismiss them, and that the amounts involved will far exceed the sum that Dr Parkes has put aside. In the end, either the universities concerned will have to exercise the right of self-governing institutions to go bankrupt or they will have to be rescued with more public money than the government will save with the cuts now decreed.

Another conspicuous loose end is that the grants committee has delivered its advice ("instructions" is more appropriate) without saying how its decisions have been reached. Are the lucky universities those with the lowest unit costs, the best track records in research grant applications, the lowest staff-student ratios, the best records in the recruitment of students, or what? There are reasons why too detailed a disclosure of the criteria that have been used would be damaging to individual universities. As things are,

however, the committee should forgive those now unwillingly dependent on its allocations of funds for suspecting that often mere gossip or even prejudice has determined its decisions. This charge, no doubt a slur, is nevertheless inescapable. If it had been prudent, or even merely politically expedient, the committee would several months ago have invited universities to agree on the criteria by which its decisions should be made. Even if the universities had failed to agree among themselves, the result would have been a licence to do what has now been done. As things are, without having consulted in advance, Dr Parkes has put his committee in the unenviable position of being regarded by the universities — his strongest supporters — as a dispensable bulwark between themselves and the public purse.

Four other questions remain unanswered. First, is it right that decisions of such gravity for the universities should be made in the absence of a mechanism for settling policy on higher education as a whole (polytechnics included)? Second, should British taxpayers be expected to put up with apparently arbitrary limits on the provision of university education, regardless of the ability and the willingness of qualified institutions to provide it? Third, what will be the consequences of last week's decisions for the pattern and even the quality of research? Finally, is it now to be supposed that higher education has become, as educationists have become too fond of saying, "irrelevant"? These questions will be much discussed in the months ahead.

Promising Americans

The United States Administration will not lightly be forgiven for the hash it has made of plans for international collaboration in this year's budget. President Ronald Reagan's budget director, Mr David Stockman, seems just the kind of man to implement a promise "to get the government off the people's backs". It is also a technical triumph that he and his colleagues were able to produce a coherent replacement for President Carter's budget during their few weeks in limbo, between the election last November and Inauguration Day. But in the process the budget team was plainly unreasonably dismissive of the consequences overseas of its proposals. And while Congress is doing what it can to repair some of the damage, at this stage it cannot put everything to rights. Certainly Congress will not be able to exorcise the impression that overseas obligations take second place, in the evolution of United States strategy, to domestic exigencies.

The trouble is well illustrated by two very different examples from the budget for the next financial year (which begins on 1 October). First, and simplest, is the case of the overseas budget of the National Science Foundation, used largely for supporting a surprisingly modest programme of direct collaboration overseas. (That large funds are still to be spent by other agencies, the State Department for example, is beside the point.) Like the foundation's educational budget, the overseas programme was drastically curtailed in Mr Stockman's budget. Nobody seems to have appreciated that the National Academy of Sciences was counting on this part of the foundation's budget for its contribution to the International Institute of Applied Systems Analysis, of which it is (with the Soviet Union) the principal member, and from which it is required by its obligations to give a year's notice of resignation. It now seems likely that the House of Representatives has found a way of letting the foundation dispose more flexibly of its budget, so that the academy will not have to



take a begging-bowl around to pay its dues on 1 January, but the future of its contributions remains in doubt. Mr Stockman may have concluded that the United States derives little benefit from the work of the international institute (which is probably true) and that even the institute's disappearance would be welcome (which may also be true). But international obligations cannot be overturned as easily as that. If they were formal treaties, duly ratified by the Senate, no budget director would think of striking out the associated costs. Do agreements on scientific collaboration deserve less careful consideration?

The trouble about the United States commitment to the International Solar Polar Mission is a different case. This potentially valuable scientific exploration of the Sun away from its equatorial plane has been planned for the past several years between the European Space Agency and the National Aeronautics and Space Administration, each of which was to have launched a satellite. There were howls of protest from Paris when Mr Stockman's budget omitted this item (see *Nature* 5 March, p.1) and science administrators in Western Europe have since fallen into the habit of complaining to their opposite numbers from the United States whenever they encounter them. Indeed, the Europeans are in danger of making too great a fuss; what is at stake is a single if expensive project, not an understanding affecting the survival of an institution, and in any case a single satellite in a polar orbit about the Sun at the next solar maximum will be a lot better than none. Even here, Congress is doing what it can to help (see page 102). It remains to be seen how the issue will be decided — but surely there are no grounds for flirting with the resuscitation of the Halley mission (in which every agency in the world capable of launching spacecraft will be participating) while a smaller sum of money would help to fulfil an international promise.

From the frying pan ...

The battle for the control of the British telecommunications network is almost over; there is a decent chance that the bill with which Parliament has been struggling for the best part of a year will become law in the next few weeks. But the war is only now beginning. In the past few weeks, several groups of private interests have been cheekily staking claims to set up in competition with the telecommunications monopoly. Cruelly as it must seem to the public monopoly (trendily known as British Telecom), other British public monopolies and nationalized industries are prominent among the would-be vultures. Thus British Rail (a wholly owned nationalized industry) and British Aerospace (wholly owned until nearly half its shares were sold off earlier this year) are talking of using the railway network as the basis for a competing trunk telecommunications network. Even more cheekily, Cable and Wireless, the state-owned company originally set up to operate telephone networks overseas, is talking of entering the domestic market even though it has not yet been sold off to the public (as the government intends). Yet British Telecom is plainly not intending to put up with whatever indignities the months ahead may bring. Some weeks ago, it put out a sombre warning of the dangers of connecting to the existing telephone network terminal equipment supplied by others than itself. Last week, it published a much more intelligent document — a reply to the study commissioned by the Department of Industry from Professor Michael Beesley, which concluded that no great harm would be done, but rather good, if private interests were allowed to rent circuits from British Telecom and to use these for providing services that could then be resold to others (see *Nature* 23 April, p.619). The argument will now begin.

The resolution of these and related issues is crucially important for the future use in Britain of a technology as full of promise as politicians have been saying this past long year. And what happens in Britain may even be instructive elsewhere; experience in the United States of the erosion of the Bell System's monopoly is not everywhere valid and is in any case confusing, while governments elsewhere in Western Europe are in much the same state of indecision as afflicts the British government. But the

British experience is likely to be less helpful than it might have been. The most reprehensible feature of the Telecommunications Bill is that nobody will be allowed to chip away at the British Telecom monopoly without the consent of the Department of Industry. In practice, it will be for civil servants and British Telecom between them to decide what shall be allowed and what prohibited. Thus although the present government has promised that within three years others than British Telecom will be entitled to sell terminal equipment to telephone users (and the illicit trade is already gathering strength), a change of government could change all that. And the latest document from British Telecom, intelligent though it is, demonstrates again what has been clear all along — that there is an urgent need for an explicit set of rules and some independent tribunal for administering them.

What Beesley said was simple. British Telecom would not come to a sticky end if it were required to follow the practice forced on the Bell System in the United States, and to lease trunk circuits from its own network to private operators who might then resell telephone services. Everybody's model is the American company MCI Communications Inc., whose customers are able to make trunk telephone calls between many (but not all) cities in the United States for roughly half the cost charged by Bell. Beesley acknowledged that such arrangements would rob British Telecom of revenue but not necessarily of profit, and argued that the business is not, in any case, a zero-sum game — symbiosis between the rump of the monopoly and private operators is just as likely as out and out parasitism of British Telecom. British Telecom protested at the time that Beesley's recommendations would "skim the cream" from its own business, and repeats that argument in last week's document — but in an especially revealing way. It repeats its fear about the cream, asserts that leasing its network to others would not improve the technical quality of services provided to customers (which is untrue) and goes on to say that such arrangements would compel it to "rebalance its tariffs in order to make individual sectors pay for themselves". The simple answer to the last complaint is "Whyever not?" But British Telecom is counting on the unwillingness of British politicians to fall in with "rebalancing" if the result means higher charges for any important sector of the British electorate.

The government has only itself to blame for the embarrassment it will now be caused. British Telecom, in its reply to Beesley, is disarmingly open about its present practice. It charges extra for use of the trunk network so as to subsidize local telephone calls and rural telephones. It urges the "material benefits" of providing a uniform service at uniform prices, and says that one of the consequences of liberalization would be increased rentals for telephone lines, "hitting hardest at the less well-off customers who make the fewest calls". Just in case the government fails to get the point, British Telecom guesses that the cost of a telephone call from a public coinbox would increase fourfold. Politically, the argument is telling. It is, however, false. The annual accounts of British Telecom are at present most obviously distorted by the government's requirement that the monopoly should finance the development of its network mostly out of current revenues. If the government decided otherwise (as it should) all tariffs could be reduced. But there is in any case no justification for the present distortion of telecommunications tariffs by British Telecom's self-assumed social obligations. Why should the interests of the telephone users "who make the fewest calls" increase the telecommunications costs of more serious users, mostly from business and industry? British Telecom should instead be required to follow economic pricing policies — charges for different services related to their marginal costs — and Parliament then invited to pay for socially valuable but otherwise uneconomic services (as is already done with railway services). The difficulty is that none of this can be accomplished without an independent and public examination of what the costs should be. The government itself is not sufficiently expert and is politically too suggestible. Thus the ironical outcome of the first serious attempt to liberalize the British telecommunications monopoly is likely to be to put the clock back ten years, and to throw the management of these vital services back into the political arena.

Congress stirs non-proliferation row

Testimony on Iraq raid backfires

A serious row affecting the future of the Non-Proliferation Treaty has broken out between the United States government and the International Atomic Energy Agency in Vienna. At issue is the appearance before two congressional committees in the past two weeks of a defector from the agency's safeguards inspectorate, Dr Roger Richter (33). At a meeting of the board of the agency in Vienna this week, Dr Sigvard Eklund, director-general of the agency, said that Dr Richter's evidence to the Senate and House committees on Foreign Relations (on 18 June and 1 July respectively) had involved the disclosure of confidential information in breach of his contract of employment, and that the agency was taking legal advice.

Dr Richter's appearances have been dramatic, to say the least. According to Dr Eklund's statement on Monday, Dr Richter last showed up for work in Vienna on 15 June. The following day, Senator Alan Cranston, chairman of the Senate committee on Foreign Relations, announced in Washington that Dr Richter, having resigned for the occasion, would be giving evidence three days later. Dr Richter's resignation was received by telex in Vienna on 18 June, according to Dr Eklund; it was not, however, accepted, but Dr Richter was instead fired.

Dr Eklund in his statement said that Dr Richter had worked for the agency since February 1978, and that he had been assigned to the section of the agency concerned with supervising safeguards in the "south and south-east" sections of the agency's territory, including both Iraq and Israel, in March 1979. Dr Richter's evidence to the congressional committees consisted most conspicuously of the assertion that the agency's safeguards were not adequate to detect violations of the Non-Proliferation Treaty by Iraq.

Dr Richter was dissuaded by Senator Cranston from quoting from a letter he had written a year ago to the US State Department, in which he had alleged that "the IAEA safeguards are totally incapable of detecting the production of plutonium . . ." such as that destroyed by the Israeli raid on Tamuz on 7 June.

One of the reasons why the agency has taken umbrage is that neither house of Congress has taken its denials seriously. On Monday, Dr Eklund told his board that the authorities in Iraq had been approached immediately after the Israeli raid on Tamuz

and visited the site on 18 June. They were unable to visit the main reactor because of the extent to which it had been damaged (and the insistence of the Iraqi authorities on a personal accident indemnity). The associated research reactor and the stockpile of enriched uranium was however inspected and found in order.

For the agency, the incident obviously raises serious questions about the fiduciary responsibilities of its safeguards inspectors. One requirement of the Non-Proliferation Treaty is that information gathered by inspection teams should be kept confidential. The fact that a defecting inspector should have told all to Congress on the day of his formal resignation is a serious blow to the system.

In the United States, Dr Richter's evidence to the Senate and the House has had an equally profound effect, and may impede the Administration's declared intention of liberalizing restrictions (made necessary by the Carter Anti-Proliferation Act) on the export of nuclear technology. The nuclear industry has been especially critical of the act's requirements that

recipient nations, even those that had signed the treaty, should go further than merely accept the Vienna safeguards before becoming eligible.

Part of the reason why the Carter Act has been controversial among potential recipients of United States' nuclear technology is that one condition for their signature of the treaty, in the early 1970s, was the promise that nuclear powers would assist with the development of peaceful nuclear technology.

Opinion is divided in Washington about the strength of Dr Richter's testimony.

In Congress, however, Senator Charles Percy, chairman of the Senate Foreign Relations Committee, says he has been shaken by Richter's evidence.

The chief casualty is likely to be the Administration's determination to reform the anti-proliferation policy it inherited in February. Even President Reagan startled the nuclear industry when he acknowledged at last week's press conference that signature of the Non-Proliferation Treaty did not necessarily imply compliance.

British universities transformed by budget

A major reshaping of the British university system was decreed last week, when the University Grants Committee sent letters to each of the 51 universities in Britain giving details of their recurrent grants for the next three academic years. But the full implications of what the committee has decided will not be clear until the details have been analysed by the Committee of Vice-Chancellors and Principals, to which the universities have separately (but in confidence) provided copies of the letters they have received from the committee.

Two features of the new pattern are however apparent. By the beginning of the academic year 1984-85, the total number of students from the United Kingdom and elsewhere in the European Community is to be no greater than 249,000, five per cent less than last year (the base year for all the committee's calculations) and 7.5 per cent less than in the current academic year. And the total recurrent grant for the universities, paid on the recommendation of the University Grants Committee, will fall from £972 million in the current year to £808 million in 1983-84.

As well as publishing a general statement of what it was about, the committee last week sent individual letters to universities including what is called "advice" about the teaching activities that should be continued (and sometimes strengthened) but also, in many cases, abandoned. Most recommendations of this kind, which only the bravest universities will ignore, concern the arts or social sciences. But some universities have also been "invited" to

abandon teaching their brand of biology.

The University Grants Committee (which has no formal mechanism for dealing with enquiries from the press) is not prepared to say how its decisions about individual universities have been arrived at. It seems, however, to have sought to preserve excellence and minority areas of study and to encourage what is known as "thrift" while maintaining regional balances. Unit costs appear to have been influential in the case of the University of Bath which, while boasting of a diversified programme of studies linked broadly (and sometimes loosely) with industry, also boasts of the lowest costs per student in Great Britain, and has been the most generously treated university of all — its income is cut by merely 7 per cent.

It is also known that the committee, in making specific recommendations to the Department of Education and Science for grants to individual universities, took advice about the performance of universities in competition for research grants from the research councils. One vice-chancellor, at least, is glad to think that his university's relative immunity from impending frugality stems from his academics' success in raising more than £3 million a year by way of grants.

Vice-chancellors at the newer technological universities most severely affected by the cuts complain, however, that in its calculations of external research support the grants committee has paid too little attention to research support provided by industrial companies as distinct from research councils. They also

point, with justifiable surprise, to the committee's formal endorsement (in its letter for general circulation) for courses of study intended to foster closer relationships between students and industry and the presence of at least three former colleges of advanced technology ("universities in waiting" in the early 1960s) among those now worst hit (Salford, Aston in Birmingham and Bradford).

The delivery of the committee's letters to universities comes at an awkward time, with the summer vacation almost everywhere begun. The committee has agreed that aggrieved universities should have a right of "consultation", which will, nevertheless, have to be exercised quickly. The committee of Vice-Chancellors and Principals is hoping within the week to put together a document showing how the pattern of the university system will be changed, but even that calculation will be jeopardized by uncertainty about the recruitment of overseas students (who pay higher fees) in the next few years. Even moderately gloomy forecasts suggest that the total reduction of university income may be as much as 17 per cent when allowance is made for that deprivation.

French universities

New appointments

While Mrs Margaret Thatcher squeezes the British universities, the departure of another lady over the channel has French universities sighing with relief. Madam Saunier-Seïté, Minister of the Universities under President Giscard d'Estaing, set out to centralize power over appointments and the allocation of degrees, and to weaken the role of some of the smaller regional universities. Now, under a gentlemanly new minister of the new government, M. Alain Savary, that is being reversed.

M. Savary says he wants dialogue with the universities, and dialogue he seems bound to get. Within a few days of the election of President Mitterrand, 100 lecturers at the University of Paris signed a declaration condemning the previous minister's "scandalous" methods of making university appointments and demanding a more democratic approach. The two principal education unions, the Syndicat National de l'Enseignement Supérieur and the Syndicat Général de l'Education Nationale, also weighed in with a joint statement warning that conservative and technocratic forces were still in control of the universities, and that they would have to be overthrown.

The chief target seems to be the Conseil Supérieur des Corps Universitaires, which according to its timetable should meet this month to consider this year's new appointments to the few university posts available. The council, strengthened by Saunier-Seïté, interviews candidates and makes its decisions in private, without right of appeal, complain the Paris 100; moreover

its council is said to be predominantly conservative and to give a poor hearing to candidates offering a novel approach to teaching or unfamiliar combinations of subjects. Certainly, the council — as now constituted — is an obstacle to university autonomy, and M. Savary, while not referring to it directly, has said he wishes to restore university autonomy and to set up new decision-making methods which will be "very decentralized".

On another tack, Savary also seems set to restore some of the second and third-level degree courses whose status as such was removed by the previous minister. A partial list of approved courses for 1981–82 was released last week. It was determined almost entirely by assessment procedures set in train the previous year and, conscious of its shortcomings, M. Savary has announced that the universities are free to appeal against the decisions (where a course has been cancelled) or to make new proposals. But he has called for "a sense of self-discipline" among the professors: there is not to be a free-for-all in which every wild proposal will meet approval.

Savary also says that appeals may not last into next year. The device is a stop-gap measure. For the long term, M. Savary plans to enter "without delay" into discussions, with all who are interested, over new mechanisms for the accreditation of courses.

Robert Walgate

US biomedical research

Against the tide

Washington

Democrats in the House of Representatives have been having little success in trying to reverse budget cuts proposed by President Ronald Reagan, but they may gain a rare victory on the issue of support for biomedical research training.

Focus of the dispute is the National Research Service Awards (NRSA) scheme, which provides about 10,000 grants annually to support postgraduate and postdoctoral research workers. The Reagan Administration is proposing that such grants should no longer contain institutional support to cover general overheads at research institutions, which would mean a cut of more than 25 per cent in grant allocation. Medical schools and universities complain that without this support — about \$50 million a year — they will not be able to sustain an adequate base across all areas of research training.

The medical schools won a preliminary round earlier this year, when both houses of Congress rejected the Administration's proposal to drop institutional support provided through the awards scheme as a budget saving for the fiscal year 1981, which began last October.

Less expected was their success in the debate on the 1982 budget in the House. The defection of a number of conservative Democrats to the Senate side resulted in

defeat for proposals submitted by the House leadership, and victory for amendments presented by Republicans.

For example, the House Science and Technology Committee had proposed deleting funds for the construction of the liquid metal fast breeder reactor at Clinch River in Tennessee, transferring much of this money to research in solar energy and conservation. The full House, however, rejected this proposal, restoring the Clinch River funds and severely reducing the solar energy budget.

In biomedical research training, however, the cuts proposed for 1982 brought a stream of protests from the research community. In a letter to Representative John D. Dingell, chairman of the House Energy and Commerce Committee which has responsibility for the budget of the National Institutes of Health, 58 separate medical and research associations warned that the cuts would be "severely harmful".

The lobbying seemed to pay off. Mr Dingell's committee recommended to the full House that the NRSA budget be raised to \$194 million from the proposed \$147.3 million.

The Republican-run Senate, however, has already passed a budget bill containing the lower figure proposed by Mr Reagan. In addition, the Senate suggests an upper limit on biomedical research supported by the National Institutes of Health of \$3.7 million, a move which the medical associations describe in their letter as "arbitrary, unprecedented and unnecessary."

Negotiations now have to take place between the House and the Senate before both sides can agree on a common bill. At the same time, there is a parallel debate going on over the budget for the Department of Health and Human Services which is responsible for the funding of the National Institutes of Health.

In particular a key Senate Committee — Labor and Human Resources — is in deadlock. The committee's previous chairman, Democrat Senator Edward Kennedy, backed by other Democrats and two Republicans, is proposing an additional \$50 million for research training awards. The current chairman, Republican Senator Orrin Hatch, is opposed to the committee taking a public stance in defiance of the President's recommendations; but he has promised that if the committee approves the lower figure, he will intervene to see if it can be raised.

Medical school lobbyists, such as the American Association of Medical Colleges, intend to keep up the pressure to have the funds restored. Dr Lamont-Havers of the Massachusetts General Hospital told a meeting of the American Association for the Advancement of Science that the proposed cuts reflected "a deep bias within the Office of Management and Budget" against the biomedical research training programme.

David Dickson

UK atomic energy

Design delays

British hopes of building a pressurized water reactor (PWR) seem now to rest on Dr Walter Marshall, chairman of the UK Atomic Energy Authority, who was last week appointed by the Department of Energy as chairman of a "task force" intended to stifle squabbles about the project — and to help reduce the cost. This development is a consequence of mounting impatience among Marshall and his colleagues on the four-man "nuclear industry group" with the delays that have accumulated in the PWR programme; they put their complaints in a letter to the Department of Energy three weeks ago.

As yet, the National Nuclear Corporation — the putative constructor — has not completed the reactor design, which should have been sent to the Nuclear Installations Inspectorate in the spring. Like a schoolboy late with his homework,



Marshall the catalyst

the corporation was able to manage by the deadline only a "reference design" that omitted details of the containment vessel and the emergency core cooling system. The missing information has not yet materialized because the corporation (and the Central Electricity Generating Board, which will have to pay for the reactor) have had cold feet about the cost.

Several modifications of the basic Westinghouse design have added to the cost. The generating board has asked for relatively easy access to the steam supply parts of the system to reduce maintenance time and the radiation exposure of workers. The containment building for the reactor was intended to have a double-walled construction, some concrete shielding was intended to be extra thick and there were to have been four (rather than two) emergency core cooling systems.

However, critics of these changes say they substitute concrete and equipment for analysis. Good chemical control of the steam supply system — for example, by purging the steam supply system before

dismantling for refuelling — can reduce radiation doses to levels acceptable to the generating board, say the critics, without using more concrete. (The board has indicated that it will not accept levels higher than those at the Heysham advanced gas-cooled reactor, a relatively clean power plant.) Some American utilities already use the hydrogen peroxide method in their PWRs. So this week a team of National Nuclear Corporation and generating board scientists has flown out to inspect such "best practice" in American reactors, and make its own measurements of radiation.

Similarly, the net benefit of quadrupling the emergency core cooling system could be obtained more cheaply by looking closely at the failure rate and significance of individual components of the system. The containment building will also probably be simpler, and similar to the optional construction designed by the Bechtel Corporation which is acting as consulting engineers to the National Nuclear Corporation.

What has worried Marshall, and now apparently the Department of Energy, is that the pursuit of perfection at the National Nuclear Corporation has been time-consuming as well as potentially expensive. His task force will function not as a decision-making body but as a forum in which the designers can be shamed into making up their minds.

All of the questions to be tackled relate to safety, and most of them involve trade-offs against cost. Marshall hopes that the outcome will be a reactor with a construction cost (per kW of generating capacity) only 60 per cent of the cost of the advanced gas-cooled reactor. He may have to be satisfied with less. Although some of the refinements of the Westinghouse design may have to be abandoned, Marshall is confident that the reactor can be built within British safety criteria.

The plan now is that the final design should be with the nuclear inspectors in the autumn. With a lot of luck, it may still be possible for the government to hold its promised public inquiry on the project before the end of 1982, almost exactly a decade after the electricity board first designated the site at Sizewell for the project. Marshall's chumminess with the minister at the Department of Energy with special responsibility for nuclear energy, Mr Norman Lamont, will help to simplify the timetable.

Robert Walgate

Large Space Telescope

View from Munich

The contract for the European Coordinating Facility for the Large Space Telescope has been awarded by the European Space Agency (ESA) to the European Southern Observatory (ESO) in Garching, near Munich. The other contenders for the prize were the Royal Observatory at Edinburgh, the Institute of

Soviet second sentences

Dr Andrei Sakharov has this week issued two new appeals to scientific colleagues abroad on behalf of fellow human-rights activists.

The first is on behalf of Aleksandr Bolonkin, a mathematician arrested in 1972 on a charge of disseminating false propaganda — in fact, for having circulated the *samizdat* "Chronicle of Current Events". For this, Mr Bolonkin received a sentence of four years' prison camp and three years' internal exile, while the state Attestation Committee refused to confirm his doctoral degree. After serving his time in camp, and just before the sentence of exile expired, Bolonkin was rearrested and sentenced to a further three years in the camp. Then, a few weeks ago, just ten days before this second sentence was to expire, he was charged again.

Such reconstructions of political prisoners due for release have been frequent in the past two to three years, and many now fear that such a fate may await Dr Sergei Kovalev, whose seven year prison term (on the same charge as Bolonkin) expires next December (to be followed by three years' Siberian exile). Kovalev, whose life is reported to be in danger, is the subject of Sakharov's second appeal, which takes the form of an open letter to Dr Linus Pauling. Included in the appeal are Tanya Osipova, Dr Kovalev's daughter-in-law and a member of the Moscow Helsinki Watch Committee, who was recently sentenced to 5 years labour camp and 5 years exile, and her husband, Ivan Kovalev, against whom it is understood similar charges of subversion are being prepared. Tanya Osipova's plight, says Sakharov, is particularly serious, since she has to serve her sentence in an ordinary criminal camp, whose regular inmates traditionally bully and exploit the political offenders to gain favour with the camp authorities.

Space Astrophysics at Frascati and the Institut d'Astrophysique and the Observatoire de Paris in a joint proposal.

ESO (whose member states are Sweden, Denmark, the Netherlands, Germany, France and Belgium — to be joined by Italy and Switzerland next year) moved last year to the Munich site from Geneva. It manages the observatory at La Silla, Chile, where the main instrument is a 3.6-metre reflector.

There is more than enough room at Garching for the space telescope institute. ESO now operates an imaging processing unit, incorporating a VAX 11/780 computer. Another VAX is to be installed, along with 15 staff members, who will be expected to spend half their time on their own research and half on coordinating the European space telescope observations.

The detailed arrangements for the use of the telescope have yet to be worked out between the US National Aeronautics and Space Administration (NASA) and ESA.

ESA's decision has ruffled several British feathers. The Edinburgh proposal was based on the Starlink image processing system, which also uses the VAX computer and links six British universities. A confidential report by an ESA subcommittee, set up to evaluate the proposals, is said to have highlighted the scientific merits of the British scheme but put it in second place because the location was "far off from most member countries". Problems with pay differentials and apparent weaknesses in management and archiving were also mentioned, but these objections are dismissed by some British astronomers, who consider that a system based on Starlink would provide the best facility for European astronomers. Such a system could, however, be developed with advantage at ESO. **Philip Campbell**

Curien on top

The council of the European Space Agency (ESA) has unanimously elected Professor Hubert Curien, president of the Centre National d'Etudes Spatiales, the French space agency, to be its next chairman. In a break with tradition, the council has also elected two vice-chairmen Dr Harry Atkinson, a British delegate to ESA, and Dr H. Grage, a Danish delegate. The hope is that the three new appointees, who represent separate national interests, will between them steer the agency onto a truly European course as it negotiates its future for the next ten years.

Despite the unanimous vote, however, Professor Curien's election was not without dissent, some delegates fearing that it might give too much weight to French arguments for the further development of the Ariane launcher. Britain had argued that it was time for a British chairman, the last one having been Sir Harrie Massey who chaired the European Space Research Organisation, ESA's predecessor, in the early 1970s. But other countries feared that the possible British candidates would be too partisan. An attempt by Italy and Switzerland to bring John Adams, ex-joint-director of CERN, the European centre for high-energy physics, into the competition failed on the grounds that he is not a delegate to the ESA council. John Adams had declined to apply for the post of ESA's director-general when it became vacant last year.

In the event, the compromise has been to elect the British and Danish vice-chairmen to work with Professor Curien. With the current uncertainty over ESA's future programme, they are bound to play a more vital role than ESA chairmen in the recent past.

Judy Redfearn

US space research

Halley again?

Washington

A faint glimmer of hope that there may, after all, be a mission to Halley's comet filtered from the House of Representatives last week. The House has voted to include \$5 million in the budget to keep the project alive during 1982. But this is less than the \$25 million which scientists at Jet Propulsion Laboratory (JPL) of the National Aeronautics and Space Administration (NASA) say is necessary in the 1982 budget for the first stage of a \$350 million project.

The Republican-dominated Senate in passing a parallel bill last month did not include money for a Halley mission because it had not been requested by the Administration. Even if the proposed mission survives the compromise bill which the two legislative bodies must now negotiate, it still has to go through the appropriations process in which budgets rather than programmes are agreed.

Scientists at JPL are hoping to convince President Ronald Reagan that *not* mounting the mission would be a serious blow to national prestige, given that the European Space Agency (ESA), Japan and the Soviet Union (in partnership with Comecon countries and France) are preparing their own plans.

Dr Ray Heacock, JPL's choice as project manager for the Halley mission, said last week that the \$5 million would be sufficient to fund the project for the first three months of the next fiscal year. After that the President, if the mission is approved during negotiations on the 1983 budget for the agency, could direct NASA to reprogramme some of its 1982 funds.

The proposal has some strong supporters, particularly among those who feel that space science activities in NASA have been unfairly squeezed by the agency's preoccupation with the space shuttle.

The original plans have also been scaled down considerably. NASA had initially talked of a spacecraft which would travel alongside the comet on its way to a rendezvous with the smaller Tempel 2 comet. The latest plans are for a more modest mission using reserve equipment from previous planned missions to launch a spacecraft through the comet's tail within 600 to 1,000 kilometres of the nucleus.

There remains hope, however, that NASA may at least be able to resurrect its full participation in the International Solar Polar Mission, originally planned to fly two spacecraft in complementary orbits over the poles of the Sun. The agency's decision, at the prompting of the Office of Management and Budget, to eliminate funding for one of the spacecraft generated a storm of protest from European allies which are building the other.

The House authorization bill passed last week added \$15 million over the Admini-

stration's request to allow NASA to continue construction of its spacecraft. This decision is likely to be supported in negotiations with Senate counterparts, where ex-astronaut Jack Schmitt is responsible for overseeing NASA programmes. The recommendations from the Appropriations Committee would also permit the project to continue, and given the importance which top State Department officials have attached to maintaining international commitments, it seems unlikely that the Senate Appropriations Committee, which meets to discuss NASA's budget next week, will object.

At the same time, NASA is unlikely to accept ESA's offer to build the second spacecraft, made during the negotiations to salvage the mission. Representative Don Fuqua, chairman of the House Science and Technology Committee, said that he was opposed to this proposal, because it was unfair to expose European contractors to the vagaries of US policy, and because even though the price-tag would be lower, spending the money in Europe would still result in a loss of US jobs and profits. TRW, the company selected by NASA as contractor for its own spacecraft, is now said to have found ways of reducing its costs considerably, a move likely to increase the mission's chances of survival.

David Dickson

Trypanosomiasis

Question of breeding

Schemes for breeding cattle resistant to trypanosomiasis are to be hatched at a research institute being planned in the Gambia (West Africa) with support from international aid agencies and foundations. The objective is to throw light on why N'Dama cattle in Africa appear to be genetically more resistant to infection by trypanosomes (also the infectious agent of African sleeping sickness) than are the more common Zebu cattle, and to find ways of propagating this resistance.

Much of the enthusiasm for the new institute comes from the President of the Gambia, Sir Dawda Kairaba Jawara, who was trained as a veterinary surgeon in Glasgow in the early 1950s, and who became leader of the People's Progressive Party in 1960. A preliminary meeting was held at Bellagio last year, and a meeting in the Gambia in May this year worked out a timetable on which further decisions must be made in time for a final decision about the project by January 1982.

Among international research projects, the Gambian research centre is unusual in that the African Development Bank seems to be prepared to take the lead in providing funds. Other interested parties include the European Community and the British government, the Food and Agriculture Organization and the World Health Organization of the United Nations, and research institutes in the World Bank

network, including the International Laboratory for Research in Animal Diseases in Nairobi. A technical appraisal of the project is being carried out this month in the Gambia, and should be completed in October.

The mechanism of trypanotolerance in N'Dama cattle is far from clear. The capacity for tolerance obviously has a genetic basis, but the older cattle are the more free from trypanosomes in the blood, suggesting that tolerance is acquired in response to trypanosomal antigens — and that even N'Dama cattle might benefit from multiple vaccines. Part of the intended research programme will be to look for genetic markers for the easy identification of resistant cattle. The long-term objective is to prepare the ground for setting up breeding centres for the propagation of potentially resistant cattle.

The initial costs of the centre are estimated at \$3.5 million, with annual running costs of \$1.5 million.

Bulgarian wildlife

Shooting for keeps

Plovdiv

Bulgaria, which this year is celebrating its thirteen hundredth anniversary of statehood, has for a number of years been committed to an ecological policy aimed at restoring its range of wildlife to the level indigenous in the country a millenium ago. During the past few years, extensive herds of roe and red deer have been built up, moufflons have been reintroduced and a small herd of bison has been established based on stock remaining from a former royal park. And now, during the past month, a second herd has been formed in the national game and forestry park at Preslav; fourteen animals, including four pregnant cows, were transferred from the existing reservation and will eventually be joined by animals from Poland and the Byelorussian SSR.

This faunal re-establishment policy is part of an overall reafforestation drive. During the long Turkish occupation, which ended just over a century ago, a large proportion of Bulgaria's primeval forests were destroyed. But shortly after the establishment of the Bulgarian People's Republic in 1944, a major tree-planting programme was started and considerable attention given to conservation. During the Second World War, Bulgarian game herds multiplied virtually unchecked, so that stocks became sufficient for the new regime to incorporate into its forestry policy a role for the human gun-bearing predator.

According to a report presented last month at the Plovdiv symposium on "Wildlife and the Environment" (part of the "Expo-81" World Hunting Exhibition), this policy is working well. Although every adult Bulgarian citizen is entitled to join the Hunting and Fishing

Union, thereby acquiring the right to hunt game, stocks are flourishing. The latest census gives 14,000 red deer (which have spread to almost all suitable habitats throughout the country), some 117,000 roe deer, which have moved permanently into arable areas and established a field ecotype, some 30,000 wild boar, 2,700 fallow deer, 2,000 moufflons, 1,500 chamois and 700 bears.

However, some small game species have suffered, apparently because of changing ecological conditions. The hare population, in particular, suffered a considerable decline in the late 1970s. The Hunting and Fishing Union, however, implemented a strict conservation plan — which included a total ban on hunting for two years — and the latest figures show a considerable increase.

Managing forests as a combined hunting and conservation resource does not seem to produce major conflicts of interest in Bulgaria. The various hunting clubs affiliated to the Hunting and Fishing Union are assigned specific tracts of the state forests, and are expected to assume many of the traditional duties of the gamekeeper — including the distribution of fodder in hard winters. No paid keepers are employed — the club members perform such tasks on a voluntary rota basis, which gives them a good insight into the problems of forest management. (Foreign tourist hunters, of course, are provided with the normal complement of beaters and ghillies — at the cost of some £500 per week.)

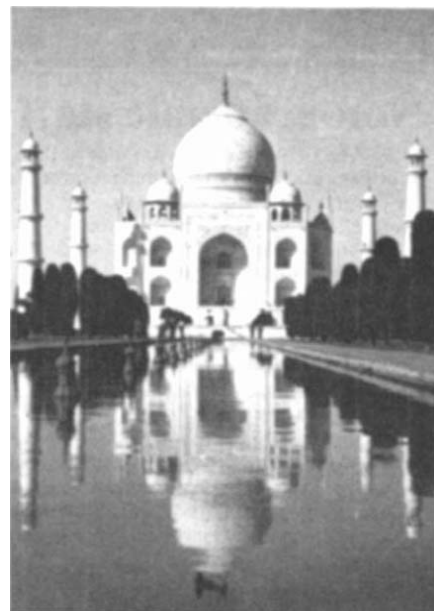
Nevertheless, Bulgaria has not been free from ecological mistakes in recent years, and this makes experts wary of introducing totally new species. Some years ago, for example, it was proposed to introduce American trout alongside the native Bulgarian variety, with which, it was confidently predicted, they would not compete. In the event, however, the newcomers attacked and virtually wiped out the local trout. Nevertheless, Bulgarian fisheries experts are prepared to consider innovations, and have reported some success with the bester — a non-migratory hybrid of beluga (white sturgeon) and sterlet, first produced in the Soviet Union about ten years ago. **Vera Rich**

India's environment

First steps

New Delhi

To protect the world-famous Taj Mahal, the Indian government is to close two coal-fired power stations in Agra, about 85 kilometres from the capital, New Delhi. This is one of the first concrete actions of India's new Department of Environment, aimed at saving the seventeenth century white-marbled monument from being blackened by smoke billowing out from the power stations. The new department has also persuaded the railway authorities to switch from coal to diesel power in their Agra workshops.



Agra's penance

These actions seem to be only a beginning for India's environment programme. According to the Ministry of Agriculture, some 175 million of the country's total 304 million hectares of land are subject to environmental problems. These include serious water and wind erosion over 150 million hectares, and shifting cultivation, waterlogging, saline and alkaline soils in the remaining area.

India is annually losing more than 6,000 million tons of topsoil through water erosion, and the total area subject to periodic floods now stands at 40 million hectares, an increase of 100 per cent in the past 10 years. Soil erosion is causing premature silting-up of tanks and reservoirs in which India has invested a massive \$12,500 million.

Another major problem is the large-scale deforestation in the Himalayas and other hilly areas of the country. And the 19 national parks and 202 wildlife sanctuaries covering more than 2.3 per cent of the geographical area of India are inadequate to protect the many endangered habitats and threatened species, especially as most of the sanctuaries suffer from a lack of any scientific, or any other kind of effective management.

One of Indira Gandhi's first concerns when returned as Prime Minister after the election of January 1980 was to stem the degradation of the environment, and this led to the setting up of a fully-fledged government department to tackle the problems. The government is expected to announce far-reaching recommendations shortly, but for the time being there is a campaign to create awareness among the general public on environmental matters. This summer, for the first time, "environmental camps" are being held all over the country to involve young people in tree planting schemes, and to put a similar message over to the inhabitants of the rural areas where the camps will be based.

Sunil Saraf

CORRESPONDENCE

Non-academic staff

SIR — I was disturbed by your swipe at the "ancillary staffs" in your comment on cuts in the budget for London University (*Nature* 11 June, p.442). The planned reductions in staff, in such a short space of time, show little concern for the careers and livelihoods of individuals, but are also uneconomic. The average cost to the nation of each unemployed person is estimated at £5,000 per year in lost taxes and insurance contributions and unemployment benefit paid out. This excludes the loss of purchasing power, lost VAT income and lost productivity.

You wonder whether the universities' chief purpose is academic — but the universities create, directly and indirectly, wealth. Foreign students bring money into the country — this may now be lost to Canada, the United States and New Zealand. With that go many exports, both "visible" and "invisible" (such as the "influence" which the Foreign Office has always laid great store by).

The efficiency of universities is obviously affected by lack of equipment — and also by lack of staff. Academics in general earn more than the other staffs. If non-academic staffs are reduced in preference to academics, the tasks now performed by academics will become more expensive. Their teaching and/or research time will be eaten into.

Britain spends less on pre-school education per head than any of her competitors. Since the 1972 White Paper on education, the staff-student ratio in universities has increased by 10 per cent. During a recession one should be prepared for the recovery, otherwise recession will soon return. The cutbacks in education (and other areas) may permanently damage competitiveness.

M. HOOPER
(University technician)

London N15, UK

The Tamuz raid

SIR — I do not object to the condemnation of Israel in your issue of 18 June ("Making Israel atone for Tamuz" p.523). On the contrary, I believe that articles such as these serve a useful purpose in that they strengthen and unify the world Jewish community. Moreover, condemnations are always preferable to condolences. It is remarkable, however, that a scientific journal such as *Nature* should tarnish its image by publishing an unscientific report that is replete with conjecture, ill-conceived logic, half truths and distortions.

To the question "Why should oil-rich Iraq be interested in nuclear power?", the scientific answer given by *Nature* is "Why not?". A long apologetic discourse follows rationalizing the presumed logic of creating civil nuclear technology in Iraq. How foolish! The Iraqi spokesmen themselves never bothered to contrive such a rationale for their reactor. President Hussein makes no bones about it — he wants bombs. He calls upon the world to "help Arabs acquire nuclear weapons — which are essential for world peace"; he also states that it is a "rational move for Arabs to try to acquire a bomb" — *New York Times*, 24 June, p.1.

The tears shed about the damage done to the "international reputation of the nuclear non-proliferation treaty" also amuse me. How

effective are the inspections? In the case of Iraq these were conducted on a limited basis, only by representatives of Communist bloc nations. Congressional testimony by Roger Richter, former American inspector of the IAEA (19 June 1981), shows how Iraq would have easily fooled reactor inspectors. Indeed, Iraq and some other nations are signatories of the treaty, but are we talking about responsible democratic societies? How much would it take for ruthless dictators, that suppress and murder political dissidents in their own countries, to abrogate an agreement? Perhaps it is time for the current ineffectual treaty to be replaced by a more meaningful one. We should all devote our efforts to promoting effective policies that would dictate limitation to the world arms race, and stop nuclear proliferation, rather than pontificate and lecture Israel about morality.

For the sake of future generations, the scientific community has the responsibility to do everything in its power to curb the real culprits in this case; the corrupt Western powers who are prepared to place nuclear energy at the disposal of madmen. In their hearts decent people thank the heroic Israelis for undertaking this necessary mission. Nuclear power must not be handed to demented killers.

IRVING LISTOWSKY
Albert Einstein College of Medicine,
New York, USA

SIR — Your editorial of 18 June (p.530) demonstrates clearly that *Nature* doesn't have offices in Israel. I take issue with your naive call for atonement on Israel's part for destroying Tamuz. Nations do not atone for legitimate acts of self-defence especially those committed during a declared war.

Egypt and Israel are at peace. The Egyptian nuclear programme is obviously needed for power production. For Iraq, peace with Israel is available for the asking. *Nature* should note that Iraq has been hostile to Israel since before Israel's independence. Iraq has not atoned for the mistreatment and expulsion of Iraqi citizens who happened to be Jewish.

It seems that you think that Israel should place its trust in the United Nations or the IAEA or the Big Powers or some other institution. Why? Did the UN stop the blockade of the straits of Tiran, did the United Kingdom and France obtain passage for Israeli shipping through the Suez Canal and when will Europe stop acquiescing in the Arab economic boycott of Israel? The credentials of world institutions do not call for trust.

Your editorial fails to mention the prospects for peace in the Middle East which were increased tremendously by the Israeli strike. It demonstrates that the cost of enmity to Israel is much higher than her enemies previously thought.

I realize that the cost to *Nature* of publishing a pro-Israeli editorial may be prohibitive in terms of lost advertising revenue and retaliatory terrorism but your respected journal should not dirty its image by ignoring history and simple logic to defend an attempt to destroy a nation.

PAUL ROTHBERG
State University of New York,
Stony Brook, New York, USA

No badger vaccine

SIR — M.J. Chapman (*Nature* 28 May, p.278) commented on various aspects of the Zuckerman report on badgers, cattle and tuberculosis. It would be inappropriate for me to deal with the medical aspects to which Mr Chapman refers other than to note that my reading of these parts of the report differs from his.

I should, however, like to comment on the question of badger vaccination. The only vaccine for tuberculosis currently in use is made from a modified (attenuated) strain of *Mycobacterium bovis* and is used only in humans. In trials, the vaccine has given unpredictable results and is of no value at all as a treatment for infected cases. It has been tried in cattle and found to be neither effective nor practical. Its effect on badgers, whether healthy or tuberculous, is not known.

The mass vaccination of badgers seems to me, however, to be unpromising. While BCG vaccine can be given orally, very large doses are required compared with the amount given by intradermal injection. Oral application on the scale required seems quite impracticable. Furthermore, to be effective any vaccination of badgers would in all probability have to be carried out before they had been exposed to infection. Recent work has found that 13 out of 49 young cubs removed from sets in one of the problem areas were already infected. This suggests that vaccination would need to be done soon after birth which is, unfortunately, impracticable in the wild.

Mr Chapman asks for consideration to be given to the views of "many zoologists" who doubt the basis of ministry policy. I can assure him that we are in contact with many zoologists, both directly and through the minister's consultative panel, and that the ministry's own zoologists are closely involved in the work.

It is not my impression that there is a body of informed zoologists who differ fundamentally with the policy that we are following. In this connection I refer Mr Chapman to the statement issued earlier this year by the Nature Conservancy Council in which they endorse Lord Zuckerman's advice.

W.H.G. REES
Ministry of Agriculture, Fisheries and Food,
Tolworth, UK

Elementary error

SIR — In *Nature* 16 April, p.538, in an article by Jasper Becker about accidents and problems at the French reprocessing plant at Cap de la Hague, mention is made of the hypothetical release of caesium-137 and rubidium-106 (if the cooling equipment for the radioactive waste storage reservoirs at La Hague had remained inoperative for ten hours).

Is the isotope number of rubidium perhaps in error, or the element? I do not find rubidium-106 listed in the *CRC Handbook of Chemistry and Physics*, 1980-81 edition.

KAY DREY
University City,
Missouri, USA
The (hypothetical) offending element is ruthenium-106 — Editor

NEWS AND VIEWS

A cycling index for ecosystems

from Robert M. May

I have heard it said that the River Thames passes through two people on its way to the sea. While I am sure this is an extravagant exaggeration, such recycling is typical of the flow of nutrients in many ecosystems.

Finn¹⁻⁴ and others have presented a formal scheme for characterizing the patterns of flow of energy or nutrients through a complex ecosystem. In particular, Finn¹ has shown how to compute a 'cycling index', which gives a quantitative measure of the extent to which flows recirculate within the system. This cycling index is the fraction of the total flow through the system that derives from cycling, expressed as a ratio to the fraction of the total that derives from flow straight through the system. Thus defined, the cycling index can clearly range from zero (no recycling anywhere in the system) to an arbitrarily large number (recycling overwhelming direct flow).

As derived by Finn and co-workers, these concepts apply to conservative systems at equilibrium, such that the books balance for overall inflows versus overall outflows. Finn observes, however, that the methodology may be extended to non-steady systems. The actual meaning of the 'compartments' that make up the system can vary greatly (trophic levels, species, individuals); although this certainly bears on the interpretation of results, it makes essentially no difference to the formalism.

Finn has analysed the cycling of energy in several ecosystems^{1,2}. One example is part of a marine ecosystem (exhibiting marine coprophagy) and has four compartments^{5,6}: the shrimp *Callinassa major*, the faeces of *C. major* and associated bacteria, coprophagous benthic fauna, and the faeces of the benthic fauna and associated bacteria. Here the cycling index, deriving from recycling between the third and fourth compartments, is ¹0.24. The second example describes energy flow in the Cone Spring ecosystem⁷, and has components for primary producers, detritus, bacteria associated with the detritus, detritus

feeders and carnivores. Here the cycling index is ¹0.16. The third example draws on the extensive studies of the Hubbard Brook ecosystem^{8,9}. Here there are essentially no energy loops, whence² the cycling index is zero.

Insofar as one can generalize from these three examples, it seems that energy can be recycled to a small but significant extent. Such recycling of energy is not in conflict with thermodynamic principles (energy, of course, cannot be degraded more than once), but arises because living systems can 'use' energy without degrading it. This is the case, for example, with the energy associated with structural or storage elements (such as cellulose, proteins or fats), which can either be catabolized or be used again as structural elements on transfer to the next compartment.

Finn² has also analysed the model of Likens and co-workers⁸ for the Hubbard Brook ecosystem, to estimate the patterns whereby various nutrients flow from the above-ground biomass through the below-ground biomass and the forest floor to the mineral soil and the available nutrient pools. The cycling indices suggest significant differences in the degree of recycling, with the elements K, Na, N, Ca, P, Mg and S having indices around 0.83, 0.76, 0.76, 0.62, 0.60, 0.59 and 0.51, respectively. Finn² comments that "this order does not appear to relate to whether or not the system is gaining or losing a particular nutrient. Hubbard Brook is accumulating N, S, and P and losing Ca, Na, Mg, and K". Notice that the cations (K, Na, Ca, Mg) rank in strict order of atomic size and charge, which determines mobility (the so-called 'lyotropic series'). Of these seven elements, N and P are probably limiting, K, Ca, Mg and S are essential but nonlimiting, while Na is neither essential nor limiting (for plants). It is therefore a bit surprising that Na is recycled to such an extent at Hubbard Brook; Finn¹⁰ has shown that Na cycling indices can be very small in other forest systems.

For Edmisten's¹¹ model of a tropical rain forest, Finn¹ finds the cycling index for N to be 1.78. This value, more than

double the 0.76 for N in the temperate Hubbard Brook forest, accords with the conventional wisdom about greater recycling of nutrients in tropical forests.

It is tempting to use Finn's cycling index in pursuit of other ecological generalities. We may, for example, expect to find that the cycling index typically increases as succession advances. Conversely, given that many kinds of pollution and other man-induced disturbances of mature ecosystems have effects akin to precipitating the system back into early successional patterns¹², it may be that such perturbations are characterized by decreases in cycling indices. These are interesting speculations. They may even be taken to suggest that the cycling index could be a useful diagnostic in the design of environmental impact statements.

As Finn² himself has emphasized, however, "Flow measures do not take into account residence times or storages, but only the amounts of nutrients actually moving and the way in which they move. . . . all the different processes involved in nutrient cycling cannot be captured and quantified in a single index". The cycling index is an elegant construct, having both theoretical and practical significance; but when it comes to evaluating the impact of disturbances upon ecosystems, I would be wary of attempting to distill complex arrays of information about flow patterns into any such single number¹³. □

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Robert M. May is Class of 1877 Professor of Zoology at Princeton University.

Plasma lipoproteins and cellular metabolism

from J. S. Owen

PLASMA LIPOPROTEINS are macromolecular complexes in which lipids are bound to a variety of polypeptides (the apoproteins) through non-covalent forces. Four main classes of lipoprotein are recognized, each defined by the density at which it floats in the ultracentrifuge — chylomicrons, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Although these classes vary widely in size, and in lipid and apoprotein composition, they appear to have a similar general structure: they are spherical particles with a hydrophobic core of cholesteryl ester and triglyceride surrounded by a 2 nm-thick monolayer of apoprotein, cholesterol and phospholipid molecules.

Chylomicrons transport triglyceride and other fat-soluble substances from the intestine while VLDL transports endogenous triglyceride from the liver. The triglyceride contained in these particles is delivered to extrahepatic tissues, thereby providing cells with an important energy source. Although almost all cells can synthesize cholesterol, several clinical and biochemical studies suggest that most cellular cholesterol is derived from uptake and degradation of LDL. By contrast, one function of HDL may be to transport cholesterol from peripheral tissues to its principal site of catabolism and excretion in the liver.

These lipid transport processes are complex; they depend on an interchange of constituent lipids and apoproteins amongst the various lipoproteins, whether by exchange collision or by specific transfer proteins, and the activities of lipoprotein lipase, hepatic lipase and lecithin-cholesterol acyltransferase (LCAT). Other aspects of intravascular lipoprotein metabolism being investigated include the relationship between the various HDL subclasses and the mechanism by which specific HDL particles remove cellular cholesterol (Tall and Small *Adv. Lipid Res.* 17; 1, 1980), the origin of the lipoproteins producing cholesteryl ester deposition in the macrophage-like cells of the arterial wall (Goldstein *et al. J. biol. Chem.* 255; 1839, 1980, and Schechter *et al. J. Lipid Res.* 22; 63, 1981), and the involvement of the liver in LDL and HDL clearance (Pittman *et al. Proc. natn. Acad. Sci. U.S.A.* 76; 5345, 1979). In contrast, one aspect of lipoprotein metabolism has been relatively neglected: the influence of lipoproteins on cellular function by their regulation of selected cellular biochemical reactions and by their role in maintaining a normal cell membrane lipid composition.

The interaction of circulating hormones and certain non-hormonal molecules with

specific cell-surface membrane receptors is an important method of regulating cellular metabolism. Plasma lipoproteins also appear to possess this regulatory capacity. The elegant work of J. Goldstein and M. Brown at the University of Texas, Dallas has established that cultured cells have high-affinity receptor sites which recognize the apoprotein moiety (ApoB) of LDL. Subsequent studies, principally by R. Mahley and colleagues at the National Heart and Blood Institute, Bethesda, have shown that ApoE-containing lipoproteins, including both HDL₁ (the HDL sub-class through which cholesterol may be delivered to the liver) and chylomicron remnants (the particles remaining after partial hydrolysis of core triglyceride), also bind to the same cell-surface receptor. After binding, these various lipoproteins regulate intracellular cholesterol metabolism and receptor activity, processes dependent on cellular uptake and degradation of lipoproteins.

Current evidence suggests that cells may also have additional lipoprotein receptors, specific for the apoprotein moieties and distinct from the classical 'LDL receptor', and that lipoprotein occupancy of such receptors is sufficient to modulate selected cellular functions without requiring internalization of the intact particles. These include the activity of membrane-bound enzymes such as adipocyte adenylate cyclase and erythrocyte Mg²⁺-ATPase which are stimulated by VLDL and LDL, the enhancement of lipogenesis in fat cells by VLDL and the apparent LDL-induced reduction of prostacyclin production in endothelial cells. However, the type of regulation most extensively studied has been the potent inhibition of mitogen-induced lymphocyte proliferation by the ApoB- and ApoE-containing lipoproteins (Hui *et al. J. biol. Chem.* 255; 11755, 1980). J. Harmony and associates at Indiana University have shown that several primary mitogen-induced membrane events in lymphocyte transformation, such as calcium ion and cyclic GMP accumulation and phosphatidylinositol turnover, are markedly suppressed by membrane-receptor binding of these apoproteins.

The membrane lipid composition of cells may be influenced by lipoprotein lipid composition as a consequence of the ready exchange between lipoprotein cholesterol and phospholipid (mainly phosphatidylcholine and sphingomyelin) and the corresponding lipids in cellular plasma membranes. Although cell membrane

transport, receptor and enzymatic functions are primarily determined by the protein constituents, there is considerable evidence that such membrane protein functions may be influenced by the properties of the fluid lipid bilayer matrix. A shift in the dynamic equilibrium between membrane and lipoprotein lipids, through abnormalities in lipoprotein lipid pattern, would therefore be expected to lead to changes in membrane lipid composition and so indirectly to membrane and cellular dysfunction. For example, the ratio of cholesterol to phospholipid is an important determinant of membrane fluidity, many membrane-bound enzymes depend on specific phospholipids for full activity, and phospholipid fatty acid composition may be a critical factor in secondary modulation of fluidity and in synthesis of prostaglandins.

Is there experimental evidence that this potential influence of plasma lipoproteins on cellular metabolism, through their effects on either membrane lipid composition or regulation of selected biochemical reactions, is physiologically important? Support has largely been obtained by study of those dyslipoprotein-aemias, associated with either genetic, pathological, hormonal or dietary disturbances, in which substantial changes in apoprotein and lipid patterns have occurred. In this context, reports by N. McIntyre and his group at the Royal Free Hospital, London on the cellular abnormalities associated with the acquired LCAT deficiency of liver disease are of interest. Lipid changes were found in erythrocytes and platelets and the abnormalities correlated with cellular dysfunction (Owen *et al. J. Lipid Res.* 22; 423, 1981); there was also evidence in patients with liver disease that their abnormal HDL, enriched in ApoE, could effect cellular regulation by reducing LDL uptake, by altering erythrocyte morphology and by suppressing lymphocyte proliferation. However, the majority of cells studied in such cases of abnormal lipoprotein composition have been isolated from the lipoprotein-rich environment of the plasma compartment and clearly there is a need to extend these observations to cells which have access only to the lipoproteins of the extravascular fluid. That such investigations will be fruitful is suggested by the interactions of abnormal lipoproteins with cultured cells and by the poorly understood metabolic and cellular disturbances frequently associated with those dyslipoprotein-aemias, such as primary and secondary LCAT deficiency and abetalipoprotein-aemia, in which marked changes in lipid and apoprotein composition occur. □

J. S. Owen is in the Academic Department of Medicine, The Royal Free Hospital, London.

MST radars: advanced tools for gravity wave studies

from Jürgen Klostermeyer

In 1974, Woodman and Guillén¹ published observations of winds, gravity waves and turbulence in the neutral atmosphere obtained by the powerful Jicamarca radar in Peru. The radar operates in the very high frequency (VHF) band at a frequency of 50 MHz (wavelength = 6 m) and was originally built for investigations in ionospheric heights. Their work stimulated the construction of several VHF Doppler radars which were specifically designed for studies in the mesosphere, stratosphere and troposphere²⁻⁴ (MST radars). Their ability to measure simultaneously height profiles of background winds, atmospheric gravity waves and turbulence makes them unique instruments for studying not only each process separately but also their nonlinear interactions.

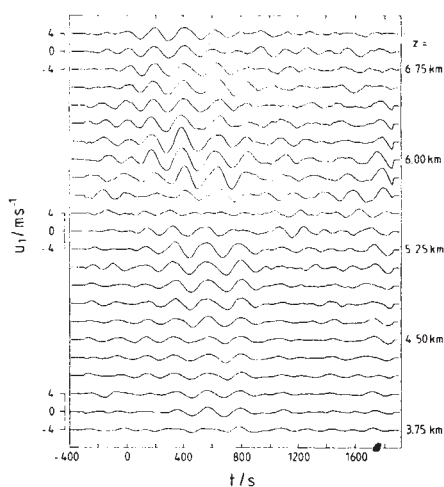


Fig. 1. Line-of-sight velocity oscillations at heights between 3.75 and 7.05 km produced by a Kelvin–Helmholtz instability during a jet stream passage. Sliding scales on the left indicate velocity amplitudes up to 3 m s⁻¹. Parameters on the right denote the heights at which the velocities were measured. The measurements were made by the German SOUSY MST radar with height and time resolutions of 150 m and 10 s. (From Klostermeyer and Rüster¹⁴.)

The radar echoes are produced by both scattering from refractive index structures with scales equal to half the radar wavelength and partial reflections from refractive index gradients. Refractive index variations are due to changes in temperature, pressure, humidity and concentration of free electrons. Humidity changes are most important in the troposphere whereas free electrons play a major part only during daylight hours at mesospheric heights above some 50 km. In the troposphere and stratosphere, the radar echo power decreases strongly with height due to the exponential decrease of

the air density whereas in the mesosphere, the free electrons give rise to a small local power maximum. The small radar scattering cross-section of the turbulent fluctuations requires extremely sensitive radars with operating frequencies preferably below 50 MHz⁵. However, depending on daytime and mode of radar operation, there is always a more or less extended height range around the stratopause refusing the onslaughts of even the most potent MST radars.

MST radars are phase coherent so that transmitted and received signals can be compared to get both amplitude and phase of the signals returned from any height. The usual procedure is to find the echo power density as a function of the Doppler shift from the transmitter frequency. This Doppler spectrum contains the necessary information about the intensity of turbulent refractive index fluctuations at scales equal to half the radar wavelength, the mean velocity of the scattering volume in the direction of the antenna beam (line-of-sight velocity) and the distribution of random velocities within the scattering volume¹. The total velocity vector can be obtained by changing the direction of the antenna beam. The temporal and spatial resolutions depend primarily on the signal-to-noise ratio of the radar echoes, optimum resolutions are a few seconds and a few tens of metres⁶.

Due to their excellent range and time resolutions, MST radars offer unique opportunities for studying the role of gravity waves in various atmospheric processes. Radar records show that gravity waves with periods between a few minutes and several tens of minutes are generally present at all heights below 100 km. At Jicamarca, several experiments using two or three antenna pointing directions were performed to measure both wave periods and horizontal and vertical phase velocities⁷⁻⁹. The results show dominant periods of between 5 and 10 min, the range of the Väisälä–Brunt resonance period in the atmosphere below 100 km. The majority of the waves propagates horizontally indicating that there is no vertical energy transport. This suggests wave sources within the observed height range rather than sources far below or above it.

A few radar experiments indicate that there are height levels at which the horizontal phase speed of observed gravity waves matches the wind speed. A wave encountering such a 'critical' level

transfers momentum and energy to the wind or vice versa depending on the strength of the vertical wind shear. In the presence of strong wind shears, a gravity wave reflected from a critical level may be stronger than the incident one. Within the

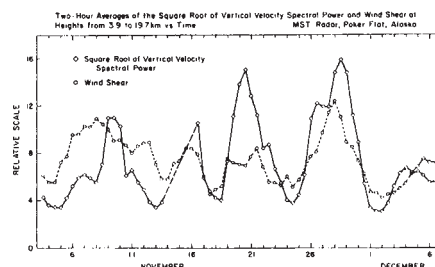


Fig. 2. Time variation of square root of gravity wave spectral power and vertical wind shear. The gravity wave spectrum was obtained by the Poker Flat MST radar in Alaska with height and time resolutions of 2.25 km and 1.5 min in the height range from 4 to 20 km. The wind shear data were derived from radiosonde ascents at Fairbanks, Alaska (From Ecklund *et al.*¹⁵.)

limits of available information, this process of 'over-reflection' could be observed by the Jicamarca radar¹⁰. Another process closely related to over-reflection is the Kelvin–Helmholtz (K–H) instability: in regions of strong wind shear, small perturbations in the meteorological noise may grow exponentially with time and rise far above the initial noise level. The horizontal phase velocity of the resulting wave matches the wind speed somewhere within the shear region so that there is again a critical level providing energy transfer from the wind to the wave. In the upper troposphere, polar front jet streams have strong shears and thus act as gravity wave sources via the K–H mechanism¹¹⁻¹³. Oscillations of the line-of-sight velocity at several heights which were generated by the K–H mechanism at the bottom side of a jet stream are shown in Fig 1. The amplitude varies strongly with height and has two maxima near 5 and 6 km, whereas the phase is almost constant in the lower and upper regions. The critical level is at 5.55 km and is characterized by a marked amplitude minimum and a rapid phase change with height.

Gravity waves have horizontal wavelengths from a few kilometres up to a few 100 km and thus cannot be resolved on the grid scales of forecasting and general circulation models. Since they play an essential part in the redistribution of momentum and energy from large-scale to small-scale atmospheric motions, proper methods have to be developed to parameterize their effects in terms of large-

Jürgen Klostermeyer is at the Max-Planck-Institut für Aeronomie, Germany.

scale motions. This task requires, among other things, research on the synoptic variability of gravity waves. An important step in this direction has recently been taken by Ecklund *et al.*¹⁵ who used part of the yet unfinished Poker Flat MST radar in Alaska to demonstrate that gravity wave activity is strongly controlled by propagating planetary waves. Whenever intense baroclinic zones moved across the radar site, the power of the gravity wave spectrum at tropospheric heights increased significantly. Intense baroclinic zones in turn are associated with strong winds and wind shears pointing towards K-H instability as a major source mechanism. Figure 2 shows the variation of the square root of the gravity wave spectral power along with the mean vertical wind shear for a period of 34 days indicating a good correlation between both quantities. This strongly suggests that K-H instabilities affect the life expectation of synoptic disturbances by continuously borrowing from their energy and momentum budgets.

Future applications of MST radars will

be directed towards more intensive studies of sources and sinks of gravity waves and their interaction with large-scale and small-scale atmospheric motions. Preliminary investigations during thunderstorms indicate, for example, that fast-rising air parcels in cumulus clouds can penetrate through the tropopause into the convectively stable stratosphere, thereby exciting a broad spectrum of gravity waves¹³. At heights below 100 km, genesis of turbulence is probably the most important wave sink besides absorption at critical levels. Radar observations show that strong bursts of turbulence arise

whenever a gravity wave generates convectively unstable regions¹⁴. Such bursts are probably identical with clear air turbulence that occasionally threatens aircrafts. In considering the stability of gravity waves we arrive at the problem of parametric instabilities. Waves with finite amplitudes so small that shear or convective instabilities do not occur should enhance a spectrum of disturbance waves by their rocking motions (like the excitation of a swing by a child). This is one of a variety of nonlinear gravity wave processes awaiting their discovery by MST radar experiments. □

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X-ray laser obtained by pumping with a nuclear bomb?

from Peter Knight

RUMOURS have been buzzing around the laser community for some months that an X-ray laser has been made to work by a group led by George Chapline of Lawrence Livermore National Laboratory. Initial reports in the February 23 issue of *Aviation Week and Space Technology* (stressing military applications) have been followed by notes in the April and May issues of *Laser Focus* that the Livermore group tested a device pumped by a small nuclear bomb at the DoE Nevada Test Site and produced a pulse of several hundred terawatts of X rays at 1.4 nanometres. So far no official comment or confirmation has been made by the Livermore. As the correspondent of *Laser Focus* points out, 1.4 nanometres corresponds to the K α line of neon. Early reports that nanosecond pulses were produced seem unlikely since such a device would probably operate as a single-pass self-terminating amplified spontaneous emission system limited at least by the picosecond upper state lifetimes. Picosecond or femtosecond pulse lengths would be more reasonable. The rumoured experiment has attracted much interest and a full account is eagerly awaited. Past efforts in this field have not survived careful scrutiny. Since a nuclear bomb is necessary in the present case, it seems difficult to check by independent experiment.

It is very difficult to create the right conditions for X-ray laser action. No mirrors are envisaged and single-pass gain

must be high enough that spontaneous fluorescence is efficiently amplified by stimulated emission during its transit down a rod-shaped inverted medium. A gain of about 100 dB is required according to G. Chapline and L. Wood (*Physics Today* June 1975, p.40) and this requires inversion densities $N^* > (\Delta\nu/L) \times 10^{18} \text{ cm}^{-3}$ where $\Delta\nu$ is the bandwidth in electron volts and L is the length of the inverted medium in centimetres. 'Cold matter' has an Auger bandwidth $\Delta\nu \sim 1 \text{ eV}$ and enormous pump intensities are required to reach such large values of N^* . In a plasma, $\Delta\nu$ is governed by Stark widths and then $N^* > 5 \times 10^{19}/L \text{ cm}^{-3}$ which requires fractional inversion $N^*/N \sim 10^{-3}$. If pump intensities are such that $N^*/N < 10^{-3}$, then one way out is to use material of more than solid density obtained through some compression scheme. It is interesting to note in this context that the Livermore experiment was supposed to be pumped by a nuclear bomb.

Many schemes for producing X-ray population inversion have been studied. Most use very high-power laser excitation of plasma targets. Promising results using this route have been reported by workers at the University of Hull (D. Jacoby *et al.* *Opt. Commun.* **37**; 193, 1981) with the Balmer α line of Carbon VI at 182Å. An early proposal due to M. Duguay and P.

Rentzepis (*Appl. Phys. Lett.* **10**; 350, 1967) suggested exploiting the frequency dependence of photoionization cross-sections to produce inversion. Tightly bound electrons are actually more easily photoionized by X rays than weakly bound electrons, so that an incident high-intensity flash of X rays could produce population inversion by leaving ions in excited states. Conventional X-ray sources are not bright enough to achieve the required gain. It is possible that nuclear explosions can produce the required rapid inversion and one might guess that this was used in the Livermore experiment.

The properties of coherent X-ray sources are so valuable that great efforts are being made to construct a useful X-ray laser device. If one were available, phase-contrast microscopy would make atomic-scale resolution of the dynamics of biological systems *in situ* possible, could solve the phase problem in X-ray crystallography and lead to the study of atomic dynamics with $\sim 10^{-15}$ second resolution. The nuclear bomb-pumped single-shot X-ray laser would be of value in scientific diagnostics but would not seem to be a route to a useful device. Nevertheless, it does represent an invigorating advance which might help in obtaining funds to continue the expensive investigation of potential X-ray laser systems. We will have to wait for more details than the leaked rumours before a proper assessment can be made. □

Peter Knight is in the Optics Section of the Blackett Laboratory, Imperial College, London.

More surprises from mitochondria

from Thomas D. Fox

MITOCHONDRIAL GENETICS began in the 1940s with the work of Boris Ephrussi on cytoplasmic petite mutants in yeast (*Saccharomyces cerevisiae*); these petites (which have extensive deletions of mitochondrial (mt)DNA) still figured in many reports at a recent Cold Spring Harbor meeting on 'Mitochondrial Genes' held in his memory. However, the study of mitochondrial genes, with or without genetics, has now been extended to a wide range of organisms. Some patterns are beginning to emerge from the comparisons, and some are not. To sum up, one might say that the functional proteins produced by mitochondrial genetic systems are generally highly conserved, while the structure, coding and modes of expression of the genes that specify them are astonishingly different in the various major groups of eukaryotic species.

Although yeast has long been the favourite organism for mitochondrial genetic studies, the best characterized mitochondrial genomes are now those of mammals. The complete human and bovine mtDNA sequences (S. Anderson, MRC Cambridge) and an analysis of transcripts from the human organelle (G. Attardi, California Institute of Technology) were reviewed. These studies and a comment on them recently appeared in *Nature* 290, April 9, 1981 and little new was left to report at the meeting. In brief, these genomes are highly compact, containing virtually no intergenic DNA and no intervening sequences within genes. Discrete transcripts appear to be cut from long precursors, generally at sites 'marked' by tRNAs, and have little or no untranslated sequence at either end. There is probably only a single promoter for transcription of each strand. The genome codes for five proteins of known function and up to eight unknown proteins appear to be coded by unidentified reading frames, or URFs. The identity and function of the URF polypeptides remains one of the major unanswered questions in this system.

A comparison of the genomes of yeast and mammals reveals very little similarity except for the deduced amino acid sequences of the five identified proteins. The yeast genome is anything but compact, with large A+T-rich regions between genes. Unlike the orderly process of mtDNA replication in animals, involving well defined start and even stop signals (D. Clayton, California Institute of Technology), there appear to be multiple origins of replication for yeast mtDNA (G. Bernardi, University of Paris; H. Blanc, Stanford University). Duplication of these origins may have helped generate the

A+T-rich DNA since Bernardi finds that origin-like sequences occur in these regions more often than expected randomly. Like replication, transcription is also more complex in yeast than in mammals, involving not only processing but multiple promoters as well. Independent primary transcripts, retaining di- or tri-phosphate 5' ends, can be detected for the regions coding the large and small rRNAs, and from at least four other sites around the genome (D. Levens, University of Chicago).

A yeast mitochondrial gene required for the synthesis of mitochondrial tRNAs has been identified and mapped (N.C. Martin, University of Texas, Dallas). The gene product is unknown, but is active in petites (which lack mitochondrial protein synthesis) and thus cannot be a protein. A plausible hypothesis here is that a mitochondrially coded RNA interacts with a cytoplasmically made protein, imported into the mitochondria, to form an enzyme (similar to RNaseP of *E. coli*) that processes mitochondrial tRNA precursors. A converse problem, that of a gene product in search of a gene, also arises in yeast, namely, where is the gene for the *var-1* polypeptide (see *Nature* 289; 119, 1981)? Although mitochondrially inherited genetic determinants cause polymorphic variations in this mitochondrially synthesized ribosomal protein, no structural gene can be found in yeast mtDNA (R.A. Butow, University of Texas, Dallas).

Several yeast mitochondrial genes contain introns, and intron-2 of the cytochrome *b* gene (*cob*) appears to code for a protein, termed maturase, which is required to splice out the intron itself (see *Nature* 289; 439, 1981). Studies on intron-4 of *cob* have revealed a similar, but rather more complex picture. First, mutations in this intron can block the processing not only of *cob* transcripts, but also those of the mitochondrial gene coding cytochrome oxidase subunit I. Second, several new polypeptides appear in intron-4 mutants, although only one seems to be directly affected by a mutation in the open reading frame (H. Mahler, Indiana University). Finally, there are *cis*-dominant mutations both in the intron's open reading frame and in the non-translatable region preceding the next exon (C. Jacq, CNRS Gif). However, one can avoid dealing with these complications if one simply makes them disappear. P. Pajot (CNRS Gif) has

done just that by isolating a spontaneous mutant in which *cob* introns 4 and 5 were both deleted, but which still made functional cytochrome *b*. This indicates that both introns were very precisely excised; where they went is unknown. Furthermore, owing to a nuclear mutation that occurred simultaneously with the intron deletions, cytochrome oxidase subunit I is made despite the absence of *cob* intron-4!

Two features of the *Aspergillus nidulans* mitochondrial genome are particularly revealing when compared with those of yeast and mammals (R.W. Davies, University of Essex). First, the cytochrome *b* gene of *Aspergillus* has a single intron, whose position corresponds almost exactly to intron-3 of the yeast *cob* gene, and whose anatomy closely resembles that of the 'maturase-coding' intron-2 of *cob*: it has an open reading frame in register with the preceding exon, followed by a short untranslatable region. Thus mitochondrial intron splicing mechanisms may be conserved, at least in fungi. Of even more interest to humans is the fact that *Aspergillus* mtDNA has two open reading frames which would code for proteins homologous to the URF-1 and URF-4 proteins coded by the mammalian genome. This finding indicates that these polypeptides are conserved beyond the animal kingdom, and raises the possibility of examining their function by genetic analysis in fungi. It also begs the question, where are these genes in yeast? Extensive sequence studies in yeast by A. Tzagoloff and co-workers (Columbia University) have not located them, and the prospects of doing so appear bleak. Are they in the yeast nucleus?

That genes have moved between the mitochondria and the nucleus is well established by the case of the ATPase subunit 9 gene, which is mitochondrial in yeast but nuclear in *Neurospora crassa* (see Tzagoloff *et al.* *A. Rev. Biochem.* 48; 419, 1979). The moving genes may leave tracks has now been demonstrated by the finding that the *Neurospora* mitochondrial genome nevertheless contains what appears to be a pseudo-gene for ATPase subunit 9: a homologous sequence with an in-frame stop codon and a region of gibberish in the middle (van den Boogaart, State University, Groningen).

The mitochondria of higher plants have large genomes (greater than 100 kilobases), synthesize more proteins than their fungal counterparts, and in some cases also contain linear plasmid molecules whose presence is associated with cytoplasmically inherited male sterility of the plants. Detailed analysis of plant mtDNA will be

Thomas D. Fox is at the Biocenter, Basel, Switzerland, but will be moving shortly to the Section of Genetics and Development, Cornell University.

accelerated by the fact that defined probes for yeast genes can be used to pick out homologous sequences by cross-hybridization (C.J. Leaver, University of Edinburgh). Protozoan parasites, yet more distant relatives of yeast, must also have homologous mitochondrial genes because yeast mtDNA coding for three cytochrome oxidase subunits and for cytochrome *b* gave specific signals when hybridized at low stringency to fragments of the kinetoplast maxicircle DNA from *Leishmania tarantolae* (L. Simpson, University of California, Los Angeles).

One aspect of genetic systems that is not supposed to evolve, the genetic code, apparently does so in mitochondria. Several differences exist between the codes used in mitochondrial genes of mammals and yeast (see *Nature* 287; 9, 1980). The only feature common to them (and to *Neurospora* and *Aspergillus*) but not to nuclear genes is the coding of tryptophan by UGA. However, it now appears that the use of UGA for tryptophan may not even be 'universal' for mitochondria, as the gene coding maize cytochrome oxidase subunit II does not contain UGA codons (T.D. Fox, Biocenter, Basel). Instead, the maize gene appears to use the codon CGG for tryptophan, in addition to the standard UGG.

Another established coding novelty in mitochondria is the ability of a number of single tRNA species with U in the wobble position to read all four codons in a family (*Nature* 287; 9, 1980). Several tRNAs, however, whose gene sequence predicts U at the wobble position, read only codons ending in A or G. In *Neurospora*, this dilemma is apparently resolved by a modification of the U in those tRNAs with restricted pairing (Heckman *et al.* *Proc. natn. Acad. Sci. U.S.A.* 77; 3159, 1980). While similar modified U residues have now been found in the corresponding tRNAs of yeast (R.P. Martin, CNRS Strasbourg), the mammalian systems must use another method to restrict pairing as direct tRNA sequencing has revealed no modified U residues at the wobble position of mammalian mitochondrial tRNAs (S. Anderson, MRC Cambridge).

Not only the evolution of mitochondria themselves was discussed at the meeting, but also the use of mitochondrial studies to trace the evolution of the organisms in which they reside. Since the evolutionary rate of base pair substitutions in the mtDNA of vertebrates is approximately ten times that in nuclear DNA (Brown *et al.* *Proc. natn. Acad. Sci. U.S.A.* 76; 1967, 1979), mtDNA provides a unique high-resolution tool for examining relationships among animals whose divergence times are relatively short. For example, a comparison of the DNA sequences of homologous regions of mtDNA from humans and four great apes allows the derivation of an evolutionary tree more firmly rooted in data (over 300 base pair substitutions) than has hitherto been

possible (W.M. Brown, University of Michigan). Studies of mtDNA restriction map polymorphisms within our own species indicate that *Homo sapiens* probably arose from a small group of individuals as recently as 130,000 years ago, although an anomalous restriction pattern from a single individual has led to speculation that 'non-sapiens' mitochondrial genomes could still be present in modern human populations (R.L. Cann, University of California, Berkeley).

Somewhat lost in the discussion of mitochondrial genes was the fact that their

expression must be largely under the control of nuclear genes. This point was brought home forcefully by the description of several nuclear mutants of *Neurospora* which fail to splice the intron from the large mitochondrial rRNA precursor (A.M. Lambowitz, St Louis University), and a nuclear mutant of yeast that specifically lacks any detectable transcripts of the *cob* gene (C. Dieckmann, Columbia University). Much more activity in this area can be expected in the future, and the fungi will again probably lead the way — at least until the complete sequence of the human nuclear genome becomes available. □

A golden age of astronomy

from Ben Zuckerman

THE latter half of the 20th century is one of the two golden ages of astronomy. This has primarily been a result of the opening up of new wave bands that extend over the entire electromagnetic spectrum. Excitement should continue with major technological advances anticipated in construction of new telescopes with larger collecting areas and improved spatial resolutions. The latter was emphasized at a meeting* entitled the "Scientific Importance of High Angular Resolution at Infrared and Optical Wavelengths".

The fundamental problem in achieving high angular resolution with ground-based telescopes is, of course, posed by the atmosphere of the Earth which distorts and distends the diffraction-limited images of all large telescopes at visual and near-infrared wavelengths. Currently, there are three main techniques for achieving high angular resolution. A. Labeyrie (Centre d'Etudes et de Recherches Géodynamiques et Astronomiques) reviewed the field of speckle interferometry. The application of this technique to optical and infrared astronomy has been pioneered by himself and his French colleagues. C. H. Townes (University of California, Berkeley) discussed multiple telescope interferometry in the infrared emphasizing the heterodyne technique that he and his research group have developed. The third technique, Michelson interferometry, has been utilized, in the optical, by D. Currie (University of Maryland) and, in the infrared, by D. McCarthy, R. Howell and F. Low (University of Arizona).

The best angular resolutions achieved to date are due to the CERGA group who have been operating a 35-m baseline interferometer in southern France and observing fringes with a photon-counting TV camera which integrates every 20 milliseconds. This system has measured stellar angular diameters and binary star

separations as small as 1 milli-arcsecond. A new 350-m baseline optical interferometer with novel concrete support structures for damping flexures may be operating at CERGA within a year's time. Baseline flexures at a level of only a few microns can obliterate the fringes. Because of phase jitter due to the atmosphere, the faintest object measurable with such an interferometer is about 10^5 times brighter than could be studied with a space interferometer of equal size. D. Dravins (Lund Observatory) described a proposed ground-based optical interferometer that would have substantially longer baselines — up to 100 km — capable, in principle at least, of achieving micro-arcsecond resolution. This would be a digital version of Hanbury Brown's intensity interferometer which has successfully measured angular diameters of bright, hot stars at the milli-arcsecond level. Here the signals from separate telescopes are mixed after detection rather than before as in all the other techniques mentioned above.

Speckle interferometry attempts to compensate for distortions due to atmospheric fluctuations by very short exposures that 'freeze' the image in a time interval that is shorter than the characteristic time scale of these fluctuations. An alternative approach to that of speckle interferometry is active optics. J.W. Hardy (ITEK) described how the figure of the primary or secondary mirror is actively controlled on a time scale that matches atmospheric changes and, also, distortions in the telescope figure induced by variations in gravity or temperature. J. E. Nelson (University of California, Berkeley) described a University of California design for a 10-m diameter segmented hexagonal primary mirror. The 36 segments, each 1.8-m in diameter, would be controlled by 168 displacement sensors, 3 tilt sensors and 108

*The meeting was held at the new headquarters of the European Southern Observatory in Munich from March 24 to 27.

Ben Zuckerman is in the Institute for Astronomy, University of Hawaii at Manoa.

displacement actuators. Townes pointed out that modern telescopes are already so complicated that these actuators do not represent a great increase in complexity.

Among the highlights of the meeting were discussions of unusual galaxies such as quasars and Seyferts by M. J. Rees (University of Cambridge) and by the chair-person of the scientific organizing committee, M. H. Ulrich (European Southern Observatory). Rees considered how measurements of the shape, location and fine structure of a quasar imaged by a foreground gravitational lens could be used to deduce the distribution of mass in the lens. In the most likely case where the lens is a massive galaxy, angular fine structure in the quasar image on a scale ranging from one arc second down to one micro-arcsecond could be used to distinguish mass contributions in the lens that range between entire galaxies and single stars. Since there is, at present, roughly 70 orders of magnitude uncertainty in the mass of the paramount contributor to the unseen mass in galaxies (ranging all the way from massive neutrinos to million solar mass black holes), such measurements are of considerable interest.

Finally, there was discussion of the future of large telescopes and, specifically, whether large single apertures (up to 10- or 15-m diameter) are preferable to somewhat smaller telescopes operating as an interferometer. As might be expected, no consensus was achieved. R. Angel (University of Arizona) lamented that the Multiple Mirror Telescope on Mt Hopkins in Arizona is the first real technological innovation in 50 years in optical astronomy. But it seems clear, from the impressive results already achieved by the CERGA group and others and by the range of novel proposals presented at the conference, that high angular resolution ground-based and space-based optical and infrared astronomy has a bright future. □

From embryo to teratocarcinoma in tissue culture

from Brigid Hogan

MOUSE teratocarcinomas are well established as a versatile model system for the study of gene expression and cell interaction during early mammalian embryogenesis. In culture, aggregates of the pluripotent stem cells, known as embryonal carcinoma (EC) cells, differentiate into embryoid bodies resembling the inner cell mass of the early mouse embryo. At first, these structures consist simply of an outer layer of endoderm surrounding a solid core of epiblast (or primitive ectoderm) cells, but many subsequently develop a quite complex organization, with various tissues derived from endoderm, ectoderm and mesoderm. However, the most dramatic evidence for the ability of EC cells to differentiate in an orderly and coherent sequence is undoubtedly their behaviour when injected into normal mouse blastocysts. Here, the cells integrate with the host inner cell mass and in the course of development may contribute towards most tissues of the chimaeric offspring.

Impressive as these results are, the degree of chimaerism obtained after injecting EC cells is unpredictable, to say the least. With some cell lines, the probability of an EC cell differentiating into normal tissues is very low, and cells which fail to do so may give rise to tumours. With other cell lines, the frequency and extent of chimaerism is much higher, although still unpredictable, and only in few tissues is the contribution more than 50 per cent. One of the most

celebrated chimaeras was "Terry Tom", a mouse in which nearly all tissues, including sperm, were populated by derivatives of EC cells of the OTT 6050 line, which had been maintained in the laboratory for more than 8 years as an ascites tumour^{1,2}. The prospect of generating more mice like Terry Tom, carrying germ cells derived not only from standard EC lines but also from teratocarcinoma cells selected *in vitro* to carry specific mutations, or new DNA sequences, ideally inserted into specific chromosomes, has inspired an immense amount of effort.

One of the ultimate goals of this work is to engineer strains of mutant mice which can act as models for human genetic diseases³, so male mice, which can transmit chromosomes to large numbers of offspring, are particularly prized. But, in spite of the fact that EC cells carrying specific nuclear or mitochondrial mutations⁴, human⁵ or rat⁶ chromosomes, or foreign DNA⁶ have been generated and in some cases injected into blastocysts and incorporated into chimaeric offspring, the colonization of the male germ line remains elusive. One reason for this is undoubtedly the fact that all the established EC lines so far used for genetic manipulation have an XO karyotype — that is, they have lost the Y chromosome during prolonged culture. For this reason, it would be very useful to be able quickly grow new teratocarcinoma cell lines from normal male embryos, insert genetic material or mutations into them, and make chimaeras, either by blastocyst injection or by the simpler technique of aggregation with monula-stage embryos⁷. Recent successes with nuclear transplantation in mouse eggs⁸ may

Brigid Hogan is in the Mammalian Development Laboratory, Imperial Cancer Research Fund Laboratories, Mill Hill, London.



100 years ago

STUDENTS of Cretaceous geology will regret to hear that Griffiths, the well-known "fossil man" of Folkestone, has been disabled for many months by rheumatism, brought on by constant exposure during the past twenty-five years, in which he has daily extracted from the wet and slippery tract of Gault clay in Eastweir Bay the remarkable series of mollusca with their pearly nacre preserved, plants, corals, crustacea, and reptilian remains that ornament not only the private collection of those who make the Gault a subject of special study, but the national museums both of this country and of the New World. In addition to collecting by far the most perfect specimens of the

Gault fauna and flora hitherto obtained, Griffiths has rendered an important service to science in carefully noting the bed or horizon from which each specimen was procured, which identification has formed the groundwork of the divisions which English geologists have been able to make in the Gault, and the correlation of these zones by M. Barrois and others with deposits occurring on the Continent. In consideration of these results, carried out by a working man under the difficulties of a struggle for life with circumstances, and the rigorous weather of the English Channel coast, it has been thought advisable to appeal to English geologists to raise a small fund which should render it unnecessary for work to be carried on when dangerous to health, and to tide him over present difficulties.

The evening *fête* of the Royal Horticultural Society was held on the 28th ult. in the Gardens at South Kensington. Coloured

lamps were disposed about the lawn, and here and there the cool plash of fountains was to be heard. The Siemens and Maxim electric lights were placed in the upper part of the Gardens, and in the lower part were two tents illuminated by the Brush electric light, and containing the plants of a flower-show, which continued next day. Brilliant effects were obtained with coloured fires behind the trees and the spray of the fountains.

There was recently landed at Marseilles a magnificent zebra which the King of Choa, Menelick II, has sent as a present to the President of the French Republic. This zebra, called the *Semaphore*, has been brought from Abyssinia by two Marseillais. The Société de Géographie, to which it was addressed from Aden, has intrusted it to the Marseilles Zoological Garden.

From *Nature* 24, 224-226, July 7, 1881.

eventually make it possible to obtain mice in which all the genetic information comes from one teratocarcinoma nucleus.

Until now, however, isolation of new teratocarcinoma cell lines has been a lengthy procedure involving transplantation of genital ridges or early embryos into extra-uterine sites such as the kidney or testis. In this environment pluripotent cells in the graft continue to grow and may give rise to tumours containing populations of EC cells which can be maintained during serial transplantation and then tissue culture. These techniques restrict work to inbred strains of mice since, for unknown reasons, tumours containing EC cells fail to develop from grafts into nude or immunosuppressed animals⁹.

Over the years, many attempts have been made to circumvent these restrictions by generating EC cell lines from embryos cultured *in vitro*. Now, Martin Evans and Mat Kaufman in Cambridge have at last been successful (see this issue of *Nature* p.154). Studies in several laboratories suggest that EC cells have more biochemical properties in common with epiblast cells in the inner cell mass of 5½-day blastocysts around the time of implantation, than with pluripotent cells present earlier or later. Normally, the number of epiblast cells is small but this increases when implantation is delayed by treating the mother with hormones. Evans and Kaufman collected batches of delayed-implantation blastocysts, allowed them to attach and spread *in vitro*, harvested the embryonic knobs containing the epiblast cells, dissociated them in trypsin and plated

the cells on a feeder layer of non-dividing fibroblasts. After a few days clumps of EC-like cells were located, dissociated and reseeded, and several cycles of such treatment yielded cultures containing predominantly undifferentiated EC cells.

The trick seems to be to provide conditions in which the pluripotent cells in the embryos are both encouraged to proliferate, either in response to growth factors made by themselves or the feeder fibroblasts, and discouraged from differentiation by disrupting cell-cell interactions. Using this technique Evans and Kaufman have isolated at least 15 new pluripotent teratocarcinoma cell lines, some from outbred mouse stocks. Those which have been analysed have a completely normal karyotype, including two with an XY genotype. It remains to be seen whether these new teratocarcinoma lines are particularly efficient at colonizing normal embryos and, in particular, the male germ line. Meanwhile, the technique opens up many exciting possibilities for exploiting the teratocarcinoma system more fully. For example, EC cells could be isolated from mouse strains carrying electrophoretic variants of X-linked enzymes, or from *T/t* mutants blocked in post-implantation development, and the biochemical properties of these cells followed during the *in vitro* development of mass cultures as embryoid bodies.

One criterion used by Evans and Kaufman to identify their teratocarcinoma stem cells is expression of a specific, cell-surface carbohydrate sequence recognized by one of the monoclonal antibodies made in humans suffering from a rare haemolytic

disease known as 'cold agglutinin disease'. These people make antibodies against different, chemically defined, carbohydrate domains of the Ii blood group antigens. The antibodies have been used by Ten Feizi and her colleagues at the Clinical Research Centre in London and Martin Evans in Cambridge as highly specific probes with which to follow the transient expression of carbohydrate sequences on the surface of different cell population during early mouse development¹⁰. These studies have now been extended by Gooi and the above workers (see this issue of *Nature* p.156) to define the carbohydrate sequence recognized by a monoclonal antibody SSEA-1 raised in mice immunized with teratocarcinoma EC cells¹¹ and to show that simple glycosylation steps involving addition of branch points or extra fucose or sialic acid groups occur at different stages of development. The significance of these subtle changes in terms of cell interactions, binding of hormones and growth factors, or morphogenesis remains to be seen.

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The ultimate computer

from Paul Davies

BLACK HOLES, it seems, get into everything these days. Jacob Bekenstein who, along with Stephen Hawking, invented quantum black holes, turns his attention to the more practical issue of telegraphy in a recent paper (*Phys. Rev. Lett.* **46**, 623, 1981). In the spirit of Shannon's celebrated analysis of the limits on information transfer through a communication channel, Bekenstein deploys some remarkable arguments gleaned from his experience with more astronomical matters.

The basis of this new departure is an unexpected relation, recently published by Bekenstein himself, connecting the ratio of entropy to energy for any physical system, with its physical size. The ratio is bounded by the dimension of the system, whether it be a black hole (an

extreme case) or a box of photons. Using the fact that information is the negative of entropy, Bekenstein turns his new relation inside out to address the problem of energy limitations on information transfer.

The transmission of information is expensive, as every telephone subscriber knows. The pressing question is whether the energy dissipation can, in principle, be arbitrarily reduced by some future technological innovations. Bekenstein says no, even if you try to get the theory of relativity to help. His result is disarmingly simple:
information rate = $(2\pi^2/h \ln 2) \times$ energy where h is Planck's constant.

Turning to an obvious application, Bekenstein considers the maximum conceivable speed of a digital computer. These days computers are so fast that designers have to worry about the speed of light being a limiting factor. We are already in the era of the relativistic computer. To beat the speed of light one

can try to make the device smaller, but then quantum theory threatens. Ultimately an over-compact computer risks imploding under gravity down its own black hole.

In a somewhat rule-of-thumb analysis Bekenstein considers the problem of computer overheating. There is an inevitable entropy generation involved in shunting any information around, and in the end this will melt the system unless it is efficiently cooled. The author considers various cooling mechanisms and their inherent limitations, and eventually comes up with the best performance criterion for the ideal computer: 10^{15} operations per second.

That is pretty fast by contemporary standards, so Bekenstein's limit will not cause too many sleepless nights in the computer industry just yet. Nevertheless, an ultimate bound on any form of technology is intriguing in its own right, and it would be interesting to see whether the idea is followed up.

Paul Davies is in the School of Physics, University of Newcastle upon Tyne.

REVIEW ARTICLE

Tempo and mode in hominid evolution

J. E. Cronin*, N. T. Boaz†, C. B. Stringer‡ & Y. Rak§

* Department of Anthropology, Harvard University, Cambridge, Massachusetts 02138, USA

† Department of Anthropology, New York University, New York, New York 10003, USA

‡ Department of Palaeontology, British Museum (Natural History), London SW7 5BD, UK

§ Department of Anatomy and Anthropology, Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv, Israel

The nature of human evolution has been viewed recently as a specific example of a more general model of evolution termed 'punctuated equilibrium'. The characteristics of this model are long periods of little or no evolutionary change (stasis) interspersed with periods of rapid (punctuated) morphological change. Careful analysis of the hominid fossil record over the past 4.0 million years, however, suggests no well documented examples of either stasis or punctuation. The evidence for the evolution of the hominid lineage is most reasonably interpreted by a model of more gradual change with periods of varying rates of evolution.

THE evolution of populations through time occurs by changes in the constitution of the gene pool. The traditional viewpoint has been that most of the change along lineages is cumulative and gradual. This hypothesis of 'phyletic gradualism'¹, proposes that if a complete fossil record of an evolving lineage were to be found, it would consist of a finely graded series of forms progressively more similar through time to descendant species and less and less similar to ancestral species. The temporally intermediate forms would be intermediate morphologically. The fact that morphological gaps appear between ancestral and descendant populations is a function of the imperfect nature of the fossil record.

This view of evolution has recently been questioned. It has instead been suggested that a substantial fraction of evolutionary change is concentrated in rapid bursts of speciation events. In the intervening periods most species exhibit slow, little, or no change at the morphological and genomic levels. The proponents of this theory suggest that phyletic gradualism plays very little part in the evolution of character states. The gaps in the fossil record are real and are the outcome of very rapid change from one form to another, thus leaving few or no intermediates in the fossil record. Speciation events occur in a short period of time, predominantly in geographically marginal populations with small effective breeding sizes. Thus the geological record is unlikely to preserve such saltations. This view of evolution has been termed punctuated equilibrium, or rectangular evolution.

Recently Gould and Eldredge¹ have argued that "a punctational view of change may have wide validity at all levels of evolutionary processes" and have cited human evolution as a particularly good example of punctuated equilibrium. They state that "no gradualism has been detected within any hominid taxon, and many are long ranging".

It is our intention in this review to show that with more fossil evidence and better dating techniques than in the past, one can clearly demonstrate directional morphological change. There are fossils that appear clearly intermediate in form and time between recognized fossil hominid taxa. We review all the examples put forward by Gould and Eldredge in support of punctuated evolution in hominid evolution and conclude that a hypothesis of phyletic gradualism is more parsimonious. Furthermore we question the degree to which genetic studies support punctuation; changes at regulatory loci or chromosomal reorganization may have rapid and pronounced effects on

phenotype but the commonly observed allozymic changes cannot clearly be related to punctational events.

Major stages of hominid evolution

Largely because of increased sample sizes and better dating (Fig. 1), there is better consensus than ever before on the major stages of hominid evolution through the Pliocene and Pleistocene. We will confine ourselves to examining the Plio-Pleistocene hominid fossil record for evidence of punctational change and not try to argue for or against the various possible phylogenetic trees presented in Fig. 2.

The origin of the hominid family is a topic of continuing research. The hominid fossil record is largely nonexistent before 4 million years ago (4 Myr) and it is therefore uncertain how Pliocene hominids and Miocene hominoids are related²⁻⁴. The lack of Upper Miocene to Pliocene fossils precludes detailed discussion of absolute rates of evolutionary change. Biomolecular results indicate a date of 4-6 Myr for the pongid-hominid split⁵⁻⁷.

The earliest good sample of undoubted hominids comes from the Laetoli Beds of Tanzania^{8,9}, dated at between 3.5 and 3.8 Myr, close to the putative hominid-pongid separation predicted by macromolecular evidence. These and later samples from Hadar^{8,9} and early parts of the Omo sequence¹⁰ in Ethiopia, and from Makapansgat, Sterkfontein and perhaps Taung in South Africa¹⁰, dating from approximately 3 to over 2 Myr, comprise the known samples of early gracile australopithecines (Figs 1, 3).

The earliest appearance of the genus *Homo* is generally considered to have been between 2.2 and 1.8 Myr^{2,9,10-12}. Specimens attributable to *Homo* are known from the Lower Member of the Koobi Fora Formation¹³, Olduvai Bed I¹⁴, Omo Shungura Members E, F and G¹⁵, Swartkrans Member 1^{16,17} and Sterkfontein Member 5¹¹ in Africa and the Djetis levels (Putjangan Formation) in Java^{18,19}. The African sample has generally been referred to as *Homo habilis* L. Leakey, Tobias and Napier 1964, or simply *Homo* sp.²⁰, while the earliest Javan hominids have been referred to as *Homo modjokertensis* von Koenigswald 1936. These two may be morphologically and temporally co-terminous^{2,10,21,22}.

Based on the close congruence in the last appearance of *A. africanus* and the first appearance of *Homo*, and on the morphological continuities seen in the two taxa (Figs 1,3), an ancestral-descendant relationship between them has been widely, but not unanimously accepted^{11,23-25}. Opposition stems

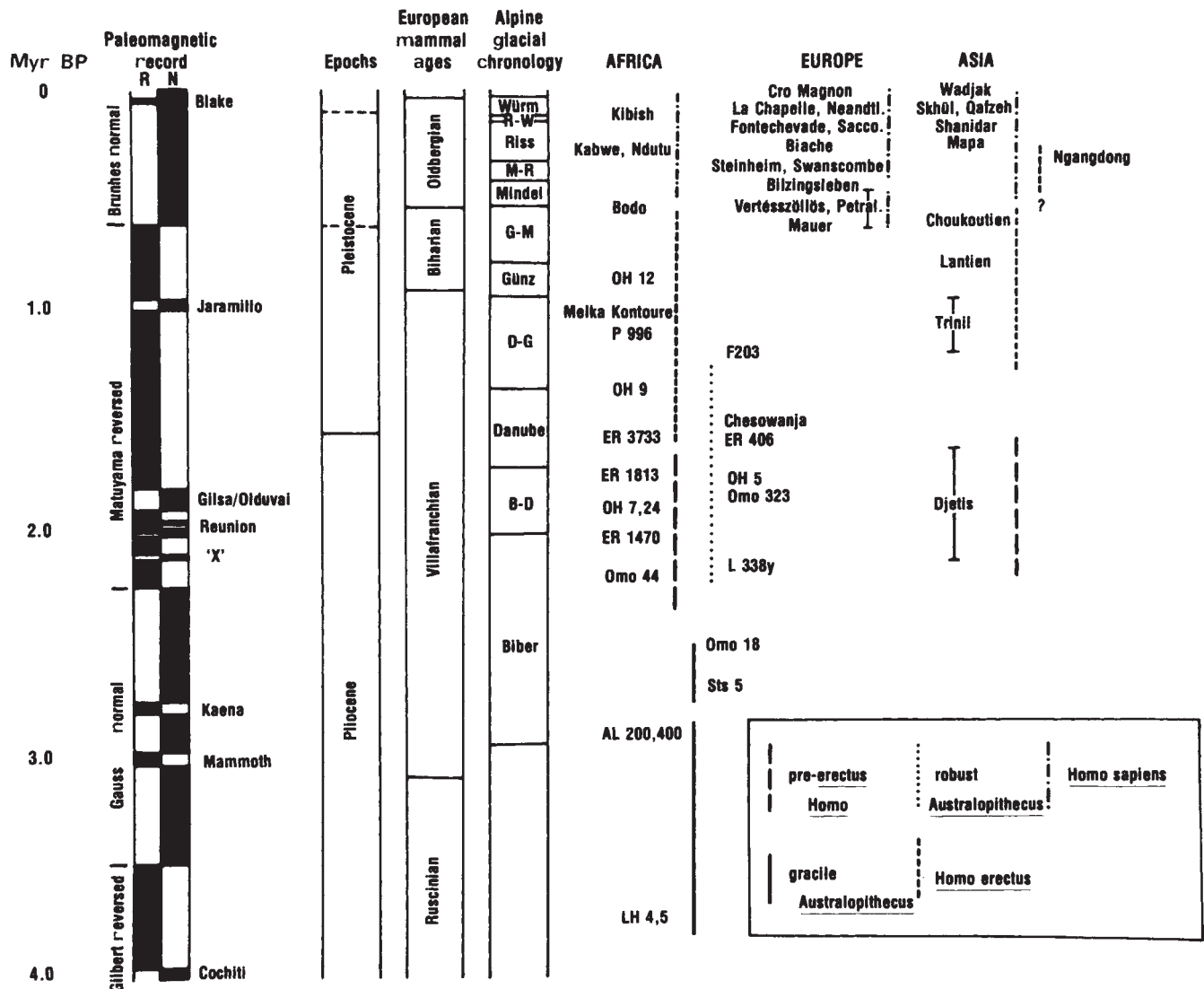


Fig. 1 Time scale and hominid lineages during the Plio-Pleistocene. Hominid fossils documenting stages along lineages are positioned according to their temporal locations. Large degrees of temporal uncertainty are indicated by error bars. The alpine glacial chronology is presented because of its historical importance rather than its accuracy. Some of the Choukoutien material may be considerably younger than shown here and in Fig. 3.

from those who believe that late surviving *Australopithecus africanus* or early occurring *Homo*²⁶⁻²⁸ preclude a simple ancestral-descendant relationship. Others divide the gracile australopithecines⁹.

Robust australopithecines were first recovered from the South African sites of Kromdraai and Swartkrans. This South African group is now referred to as *Australopithecus robustus* (Broom), 1938. The peculiar facial and dental morphology of *A. robustus* led Broom to believe that this hominid represented a side branch of human evolution.

In 1959 the type specimen of another robust hominid was discovered at Olduvai Gorge. This hominid, *Australopithecus boisei*, has since been found at other East African localities: Beds I, II and IV at Olduvai Gorge²⁹, Peninj (Humbu Formation)³⁰, Chesowanja (Chemoigut Formation)³¹, Lower and Upper Members of the Koobi Fora Formation²⁰ and Omo Shungura Members E, F, G and possibly H and L¹⁵.

The earliest appearances of the robust australopithecines in both East and South Africa seem to be at 2 Myr, and they persisted as late as 1.0 Myr^{2,32}.

The actual phylogenetic relationship of the robust lineage is widely debated. Some believe that the taxon *A. africanus* is directly ancestral to robust australopithecines⁹ (Fig. 2), with the former species showing shared derived morphological features

with the latter taxa. Others believe that this relationship has not been clearly demonstrated and that *A. africanus* provides a suitable candidate for a form that gives rise subsequently to the contemporaneous lineages *Homo habilis* and robust australopithecines².

The first remains of *Homo erectus* were discovered by Dubois in 1891. Assigned by him to the genus *Pithecanthropus*, these and subsequent discoveries from Java, China and Africa are now classified within the genus *Homo*³³. The morphology of the *H. erectus* material from Choukoutien, China (Fig. 3) was described by Weidenreich and this sample has provided the main data on the characteristics of this species³⁴⁻³⁷. Further important material has been recovered from Early and Middle Pleistocene deposits in Java, Middle Pleistocene deposits in Algeria and Early Pleistocene deposits at Olduvai and Koobi Fora³³. (Claims for the occurrence of this species in the Middle Pleistocene of Europe are overly dependent on the parts preserved in the various fossils and the characters which are utilized for classification—see discussion on the Petralona cranium below.) Recent definitions of this species have been provided by Le Gros Clark²³ and, in greater detail, by Howell¹⁰. Most anthropologists accept that *H. erectus* was ancestral to *Homo sapiens*. Specimens often quoted as displaying 'intermediate' or 'mosaic' characters between *H. erectus* and *H. sapiens* include Broken Hill and Omo

(Kibish) in Africa, Ngandong in Java, and Arago, Vertésszöllös and Petralona in Europe³³ (Fig. 3). Other recently discovered fossils which may belong to this intermediate category are the African Middle Pleistocene specimens from Salé³⁸, Ndu^{29,39} and Bodo⁴⁰. However, not all palaeoanthropologists are convinced that *H. erectus* is ancestral to *H. sapiens*^{41,42,133}.

The term *H. sapiens* has recently been used to include various Middle and Late Pleistocene fossils which can be distinguished from *H. erectus*. Campbell introduced a system utilizing subspecific names for geographical or temporal sub-groups within *H. sapiens*⁴³ and, rightly or wrongly, the subspecies name *H.s. sapiens* has become synonymous with anatomically modern humans in general. Some palaeoanthropologists have preferred the term 'archaic *H. sapiens*' for fossils considered more 'advanced' than *H. erectus* but still morphologically distinguishable from 'modern' *H. sapiens*⁴⁴ (Fig. 3). Within the former category are the 'Neandertals', placed by Campbell⁴³ in the category *H. sapiens neanderthalensis*. Others have defined 'Neandertal' more broadly to include fossils from the Old World which they consider to be ancestral to modern humans throughout the Late Pleistocene range of *H. sapiens*^{25,45}. Yet others have attempted to subdivide the Late Pleistocene material using morphological criteria and have delineated

'Neandertals' as a group supposedly restricted to the earlier Late Pleistocene of Europe and western Asia, which can apparently be differentiated from penecontemporaneous fossils from Africa and eastern Asia^{46-48,93}. The origin of anatomically modern *H. sapiens* (whether from one restricted area or more widely) is still in dispute and lack of good fossil evidence from many parts of the Old World makes resolution of this problem more difficult.

Hominid fossil evidence and rates of evolution

Gould and Eldredge¹ suggested that the gaps in the fossil record should be treated as data, as though they were 'real' and not artefacts of the fossilization process. Thus the observation that intermediate forms between taxa are frequently not known is explained on the basis that evolution from one to another species occurred so rapidly that no transitional forms were preserved. The basic assumption here is that the fossil record is relatively complete.

Available data do not support Gould and Eldredge's assumption of a complete fossil record for the Hominidae.

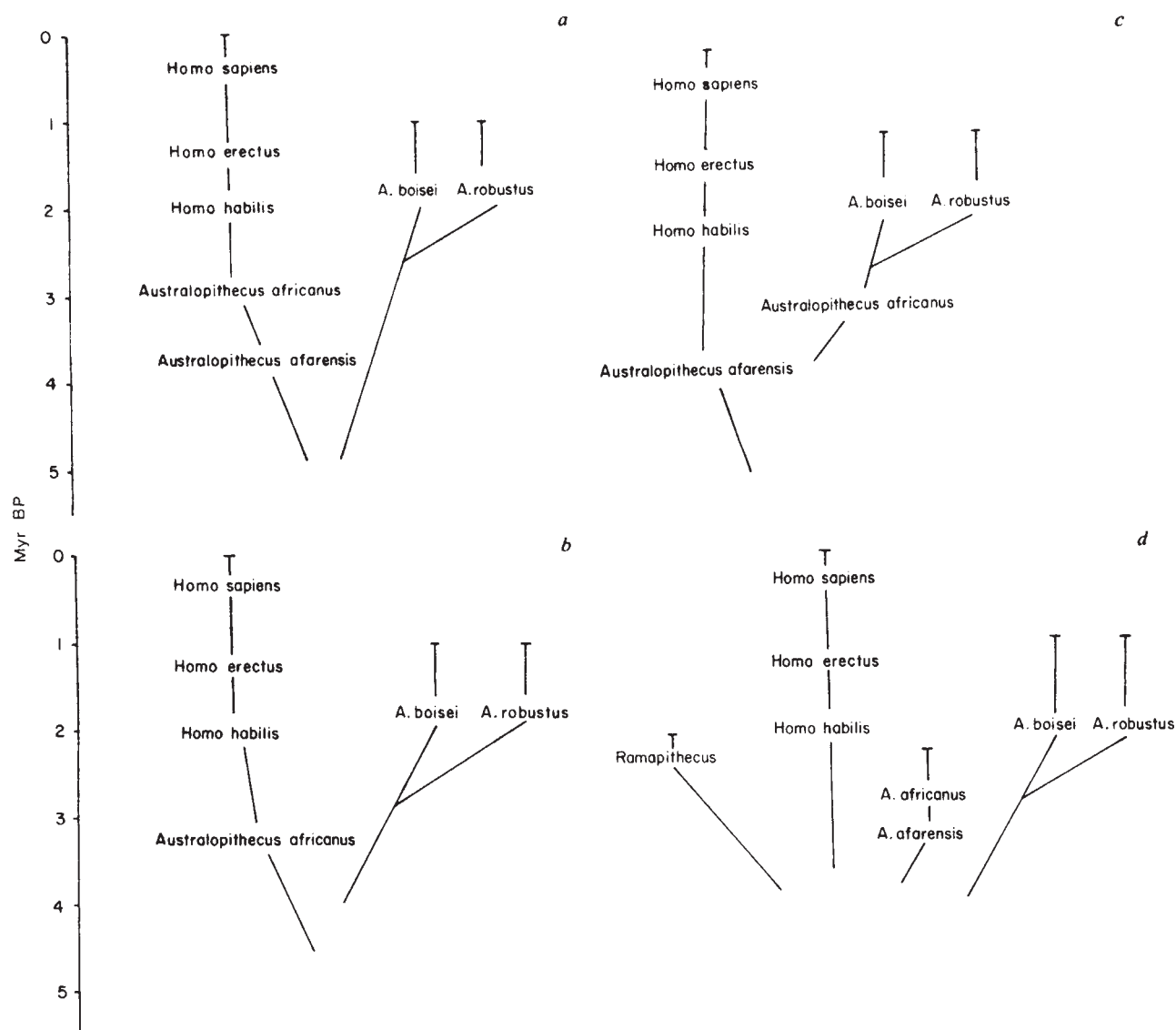


Fig. 2 Alternative phylogenies of the major hominid taxa²⁻⁴. Although not encompassing all proposed schemes, these four phylogenies are representative of the current most likely hypotheses. One of us (N.T.B.) favours phylogeny *b*, whereas another (Y.R.) favours phylogeny *c*. All of us concur, however, that *A. africanus* is not far removed from a basal australopithecine morphotype. The phylogeny represented in *d* is a polyphyletic hypothesis favoured by R.E.F. Leakey²⁷.

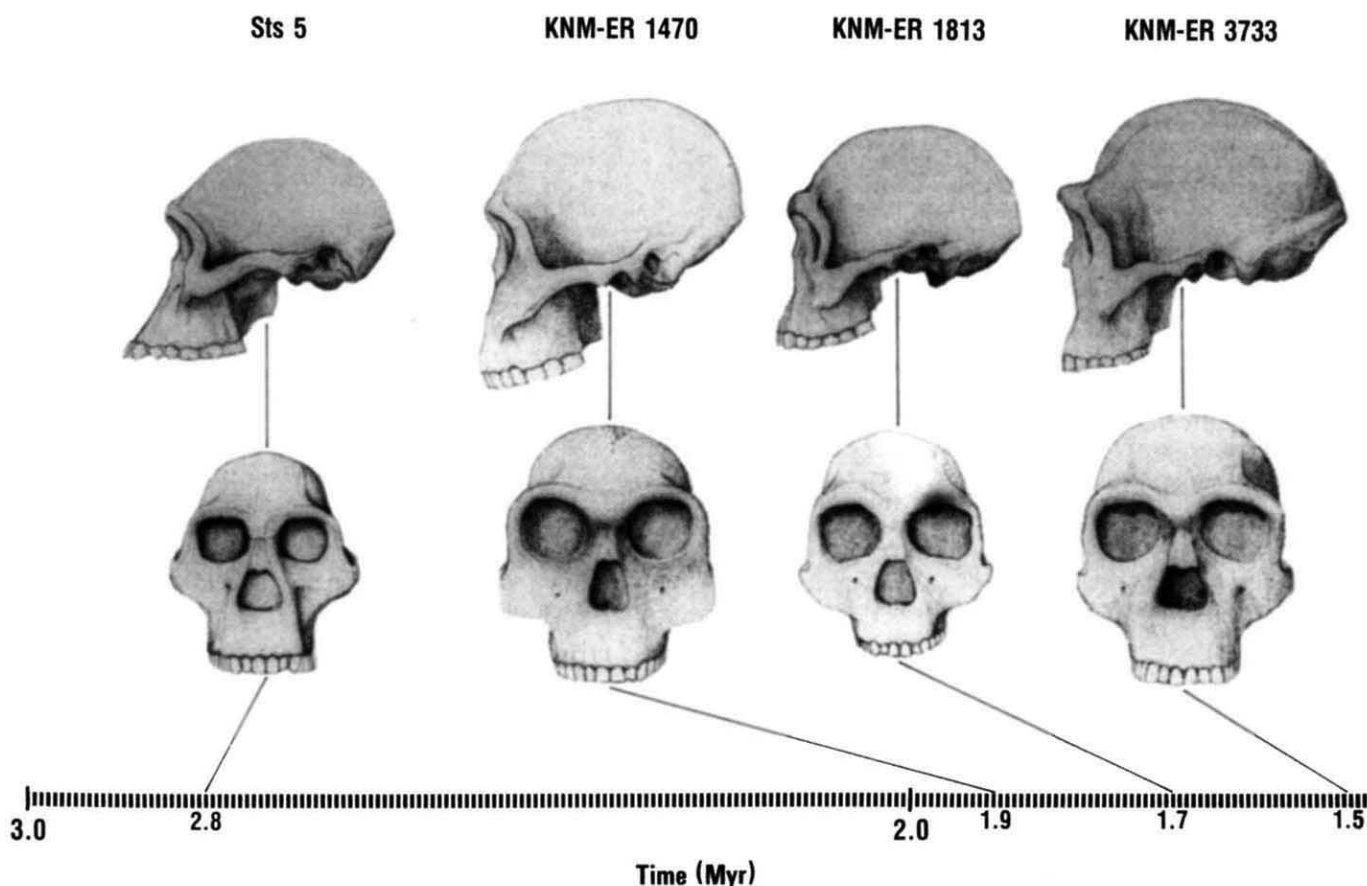


Fig. 3 Certain key hominid fossil crania in the 'gracile' hominid lineage through time, plotted along the bottom of the figure from left to right.

Behrensmeyer⁴⁹ estimates that only 4×10^{-5} of the original hominid population over a span of 1.5 Myr is represented at Koobi Fora. At Omo Shungura, with the longest sequence of hominid-bearing sediments yet known⁵⁰, 35 lithological units accounting for only 38.3% of the total vertical stratigraphical thickness have yielded hominid remains. If the total time represented by the Shungura Formation is almost 2.6 Myr¹⁰, this implies a loss of 1.6 Myr of time. Using an average of empirically derived population density estimates for Omo Hominidae (1.05 ind km^{-2})⁵¹, an area of 500 km^2 and a generation time for early hominids of 20 yr, the 215 known fossil hominid individuals from Omo Shungura derived from populations totalling 6.8×10^7 individuals. Thus, the fossil sample represents a mere 3.2×10^{-6} of the original population. In terms of both time represented and numbers of the original populations, the hominid fossil sample seems to be a very imperfect indication of the actual palaeobiology. In addition, the fragmentary nature of the preserved remains frequently does not allow phylogenetic interpretation. Thus there is almost no support for the assumption that the fossil record of hominids is complete, although a general overview of the major stages is possible while we await new fossil material.

Meanwhile recent sophisticated geochronological studies have provided sufficiently precise dates for existing fossil material to test the hypothesis of punctuated equilibrium. Two such tests are presented in Fig. 4. Means of body weight estimates and cranial capacity of the 'gracile' hominid lineage are plotted through time. The trends seem to show no jumps or discontinuities. Any impulse to draw a step diagram through the points should be resisted while the most parsimonious approach is to interpret the trends with a best-fit line.

Gould⁵² has recently suggested that the supposed long temporal range of *A. afarensis*, based on a 3.7–3.8 Myr date on Laetoli and a 2.8–3.2 Myr date on Hadar, indicates morpho-

logical stasis over time. Two considerations argue against this claim. The fossil remains from Laetoli are not abundant, and there are no diagnostic crania. Although some specimens in this sample do show dental and mandibular similarities to Hadar specimens, it is unknown to what degree the crania, which are taxonomically paramount for Hominidae, would correspond. Thus, a morphological argument for stasis cannot be sustained.

Second, while the K–Ar dating of tuffs at Laetoli and Hadar remain to be confirmed by other dating techniques, such as palaeomagnetism, there are preliminary faunal indications that Hadar and Laetoli may be closer in time than the absolute dates would suggest. Thus, a temporal argument for stasis within the proposed taxon *A. afarensis* is unconvincing.

A good case for phyletic gradualism within the gracile australopithecine lineage could be made from a comparison of South African *A. africanus*, with the more primitive and probably older *A. afarensis*. On the basis of the present samples, Hadar and Laetoli specimens evince generalized hominoid traits in having P_3 mesiobuccally set in the tooth row, large canines with distal longitudinal wear facets, I^2-C^1 diastemata and a relatively small cranial capacity⁹. They also possess certain derived traits in common with *A. africanus* from South Africa, particularly in the innominate and femur⁵³, face and cranial vault⁵⁴, and mandibular and dental morphology⁵⁵. Further fossil and geochronological evidence is needed to document the proposed transitional period from Laetoli and Hadar to classic *A. africanus*, but the balance of evidence does not support stasis within the Laetoli–Hadar samples through time nor punctuational change from these to *A. africanus*.

Two specimens of cardinal importance to the punctuated equilibrium argument come from the Koobi Fora Formation, Kenya (KNM-ER 1470 and KNM-ER 3733, ref. 20; Fig. 3; Table 1). The dating of these specimens is of paramount

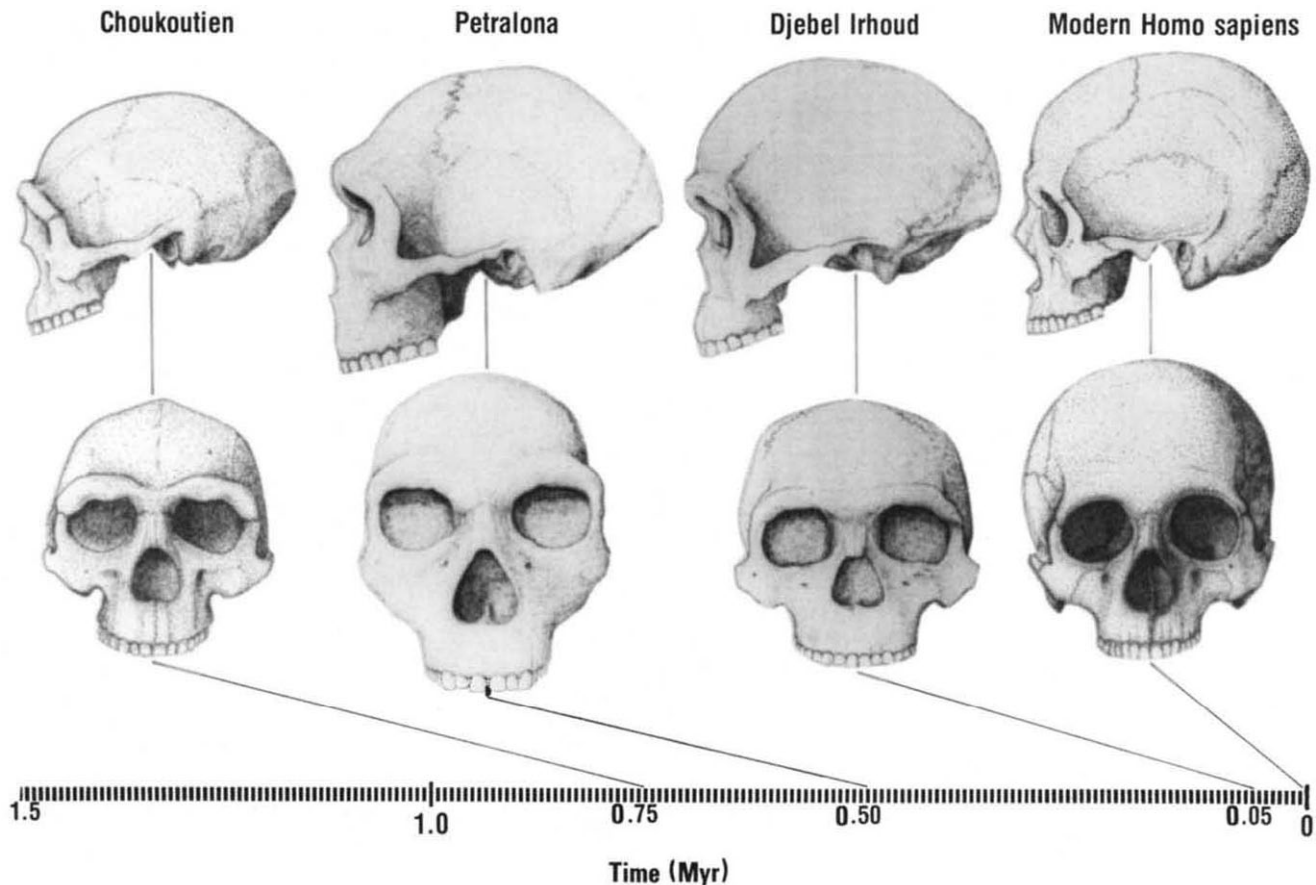


Fig. 3 (cont.)

importance but faunal and geochronological dating of East Lake Turkana had been controversial until a recent solution was reached^{56,63}.

KMN-ER 1470 comes from the Lower Member of the Koobi Fora Formation, 37 m below the KBS Tuff in Area 131 (refs 20, 57, 58), and at an unknown distance above the Tulu Bor Tuff. The specimen has been dated on the basis of $^{40}\text{Ar}/^{39}\text{Ar}$ determinations on the overlying KBS Tuff, initially reported to be $2.6 \pm .26$ Myr BP⁵⁹. Leakey⁵⁷ claimed an age of 2.9 Myr BP for the find, adding ~300,000 for deposition of the 37 m of sediment separating ER 1470 and the KBS Tuff (12.3 cm per 1,000 yr). However, faunal studies^{60,61}, K-Ar redating of the KBS Tuff^{62,63}, tuff chemistry studies⁵⁶ and fission track dating⁶⁴ have now demonstrated that the KBS-131 Tuff is only ~1.8 Myr BP and is correlative with Tuff H-2 in the nearby Omo sequence at the same date. The sub-KBS fauna, which includes ER 1470, correlates with Omo levels about 2.0 Myr BP (refs 61, 65) and this is also the most likely date of KNM-ER 1470.

KNM-ER 3733 comes from the lower part of the Upper Member of the Koobi Fora Formation⁶⁶ in Area 104 (ref. 28), having been found in a sand infilling ~10 m above the post-KBS erosion surface⁶⁷. Another specimen, KNM-ER 1813, to be discussed below, comes from the upper Lower Member of Koobi Fora Formation in Area 123 (refs 13, 20), 8–10 m below the lateral correlative of the post-KBS erosion surface⁶⁷. ER 1813 is thus stratigraphically 18–20 m lower in the sequence than ER 3733. Assuming a sedimentation rate of 10–15 cm per 1,000 yr (60 m between the KBS Tuff and the overlying Chari Tuff 1.8–1.25 Myr), the difference in time between ER 3733 and ER 1813 is between 200,000 and 120,000 yr. Probable absolute dates are about 1.8 Myr BP for ER 1813 and 1.6 Myr BP for ER 3733.

Morphological and chronological arguments have been advanced to demonstrate the evolution of the earliest *Homo* from gracile australopithecines^{2,10}. Opponents of this view have held that *Homo* co-existed with gracile *Australopithecus*^{27,68}.

KMN-ER 1470, which has now been shown to have a spuriously old date, was a key part of this argument. It is a cranium lacking teeth but showing the large cranial vault, slight mid-facial prognathism, post-orbital expansion, relatively small temporal fossae and unpronounced supraorbital tori generally associated with the genus *Homo*. However, its relatively robustly constructed face, flattish naso-alveolar clivus (recalling australopithecine dished faces), low maximum cranial width (on the temporals), strong canine juga and large molars (as indicated by remaining roots) are all relatively primitive traits which ally the specimen with members of the taxon *A. africanus*. The specimen probably represents a member of the earliest species of the genus *Homo*, *H. habilis* (or *H. modjokertensis*^{2,22}), also known in Africa from Olduvai¹⁴, Omo⁶⁹, Sterkfontein⁷⁰ and Swartkrans¹⁶. KNM-ER 1470, like other early *Homo* specimens, shows many morphological characteristics in common with gracile australopithecines that are not shared with later specimens of the genus *Homo* (Table 1); in fact, Walker^{26,28} has classified ER 1470 as australopithecine. When accurately dated, and considering the morphology, ER 1470 supports the graduated evolution of *Homo* from a gracile australopithecine species in the later Pliocene.

Various authors^{8,9,55} have recently discussed these ancestral australopithecine populations. The Pliocene specimens, whether referable to the new taxon *A. afarensis*⁸ or to *A. africanus*² (Fig. 2), and undeniably primitive in their morphology, do show many characters which link them with later *Homo* (Table 1); M. D. Leakey⁶⁸ has in fact, classified them as *Homo* sp.

The gap between *H. habilis/modjokertensis* and *H. erectus* has now been filled by several important specimens, including KNM-ER 1813⁷¹. ER 1813 shows incipient supraorbital tori depressed in the glabellar region, a post-toral sulcus, a post-toral temporal ridge (similar to OH 9), a slightly more antero-posteriorly elongated cranium than specimens such as OH 13, a slight torus occipitalis and a mid-facial region slightly more

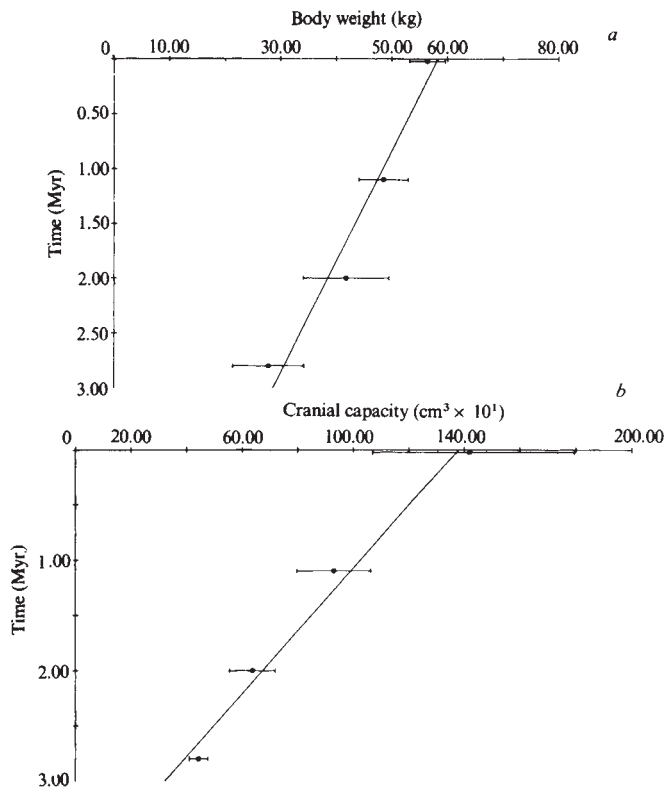


Fig. 4 a, Means of body weight estimates of hominid taxa in the 'gracile' lineage through time. The mean of *A. africanus* body weight estimates (27.7 kg) is plotted at 2.8 Myr BP and is an average of (in kg): 18.65, 25, 32 and 35.3 (refs 139, 140, 4, 141 respectively). The mean for *H. habilis* (41.8 kg) is plotted at 2.0 Myr BP and is an average of (in kg): 31.3, 40, 43 and 52.8 (refs 139, 140, 4, 141 respectively). The mean for *H. erectus* (48.5 kg) is plotted at 1.1 Myr BP and is an average of (in kg): 42.5, 50 and 53 (refs 139, 140, 4 respectively). The mean for *H. sapiens* (56.4 kg) is plotted at 0.02 Myr BP and is an average of (in kg): 52.2, 57.0 and 60 (refs 139, 140, 4 respectively). Bars represent 1 s.d. on each side of the mean. Regression line is $BW = 58.25 - 9.9t$, $r = -0.97$, where BW is body weight and t is time in Myr. The trend appears to be gradual from *A. africanus* to *H. sapiens*.

b, Means of cranial capacity estimates of hominid taxa in the 'gracile' hominid lineage through time. Times for taxa are as in Fig. 4 legend. The mean of *A. africanus* (443 cm³) is from (in cm³): 450 for AL 288-1 and 385 for AL 166-9 (ref. 54); 485 for Sts 5, 436 for Sts 19/58, 428 for Sts 60 and Sts 71, 500 for MLD 1 and 435 for MLD 37/38 (ref. 139). The *H. habilis* mean is from¹³⁹ (in cm³): 687 for OH 7, 650 for OH 13, 590 for OH 24, 752 for ER 1470 and 509 for ER 1813. The *H. erectus* mean is from (in cm³): 1067 for OH 9, 727 for OH 12 (ref. 139) and 850 for ER 3733 (ref. 79); 850 for Sangiran 1, 775 for Sangiran 2, 890 for Sangiran 3, 750 for Sangiran 4, 975 for Sangiran 6, 915 for Sangiran 7, 1,030 for Ckt 2, 915 for Ckt 3, 1,225 for Ckt 10, 1,015 for Ckt 11 and 1,030 for Ckt 21 (ref. 140). The *H. sapiens* mean is estimated at 1,420 cm³ as midway between means for *H. s. neanderthalensis*¹⁴⁰ and *H. s. sapiens*¹⁴⁰. Bars for *H. sapiens* represent observed range¹⁴⁰ and for other taxa represent 1 s.d. on each side. Regression line is $CC = 1378.6 - 352.1t$, $r = -0.99$, where CC = cranial capacity and t = time in Myr. Similar results have been obtained by other authors^{142,143}.

prognathous than ER 1470. It nevertheless retains many early *Homo* characteristics such as lack of ectocranial crests, relatively straight nasals and a small temporal fossa. Its intermediate temporal position, as discussed above, and intermediate morphology can be interpreted as linking the earlier *Homo* populations and later *H. erectus*.

One *H. erectus* specimen from Koobi Fora (KNM-ER 3733) is claimed¹ to be earlier than non-African *H. erectus*, but morphologically within the range of Choukoutien *H. erectus*. Thus similarities between KNM-ER 3733 and Choukoutien *H. erectus* are said to provide an example of stasis after a rapid evolutionary speciation event. As discussed above, ER 3733 comes from the lower portion of the Upper Member of the

Koobi Fora Formation, with a probable date of about 1.6 Myr. If we exclude the Indonesian Putjangan specimens, it is older than other Old World *H. erectus*: OH 9, Bed II, Olduvai at 1.3 Myr BP (ref. 72); P996-17, Member K at Omo at 1.4 Myr (ref. 15), Trinil at ~1.3 Myr and later¹⁹; and Melka Kontoure at ~1.0 Myr BP (ref. 73). Choukoutien is not well dated radiometrically, but has been ascribed on faunal grounds to ages ranging from Early to Late Pleistocene⁷⁴. Fauna from the main hominid levels (such as Locus L, layers 8-9) is typically Middle Pleistocene and correlative with the European 'Cromerian'. Kahlke and Chou⁷⁵ have reported *Ailuropoda*, *Macaca robustus* and *Elephas cf. namadicus*, taxa characteristic of the late Early Pleistocene and early Middle Pleistocene⁷⁶, from the lower hominid-bearing levels at Choukoutien. Kahlke⁷⁷ states that *E. namadicus* is part of the Asian Middle Pleistocene faunas which correlate indirectly with Süssenborn ('Mindel'-'Elster') faunas of eastern Europe. The Early/Middle Pleistocene boundary is now conventionally taken⁷⁸ as 0.69 Myr BP and on faunal grounds most of the Choukoutien hominids would post-date this datum. Although the entire assemblage spans a considerable time range, the main finds of Choukoutien *H. erectus* do in fact appear to be at least 0.8 Myr younger than ER 3733. On present evidence specimens attributed to *H. erectus* extend from about 1.6 to 0.6 Myr, or younger.

The available evidence does not support the hypothesis of morphological stasis in *H. erectus* through time. The peak in robusticity in the evolution of *H. erectus* did not occur with the earliest appearance of the species. The earliest examples of *H. erectus* possess a relatively longer and flatter face, shorter, thinner and less buttressed cranial vault, smaller cranial capacity, and a thinner and narrower supraorbital torus than later *H. erectus*. ER 3733, which is the earliest relatively complete example of *H. erectus*, falls outside and below the range for Choukoutien *H. erectus* fossils in four of the five measurements provided by Leakey and Walker⁶⁶. Its cranial capacity of about 850 cm³ (estimated by Holloway, quoted in ref. 79) is outside the Peking range (915-1,225 cm³)³³ and is closer to Javanese *H. modjokertensis* specimens. The supra-orbital torus appears thinner than that of later *H. erectus* specimens such as OH 9, Sangiran 17 and the Choukoutien crania and recalls that of earlier fossil hominids ER 1470, ER 1813 and OH 24. The long, flat, vertically oriented face with a deep sub-nasal area is perhaps a retained primitive feature from an ancestor similar to ER 1470. Table 1 summarizes the morphological continuities to be seen from early to late *H. erectus*, thus vitiating Gould and Eldredge's contention of stasis in this clade¹.

In the case of *H. sapiens*, Gould and Eldredge¹ imply that modern *Homo sapiens* is morphologically similar to the earliest examples of this species. This interpretation would be more defensible if they were referring only to anatomically modern man as *H. sapiens* because, for example, the earliest anatomically modern populations of Europe and Australia over 20,000 yr ago do closely resemble their present-day aboriginal counterparts^{46,80}. But even here it can be argued that noticeable evolutionary change has occurred^{81,82}. However, most anthropologists would extend the term *H. sapiens* to include various fossils from the Middle Pleistocene (such as Swanscombe, Steinheim, and Broken Hill) and the earlier Late Pleistocene (the Neandertals).

To test the alternative models of phyletic gradualism or punctuated equilibrium in the evolution of *H. sapiens* we require (as Gould and Eldredge state) reasonable samples with good stratigraphical control. The only area which approaches these conditions in terms of samples with good stratigraphical control is Europe. If phyletic gradualism is a reality and *H. erectus* was gradually transformed into 'archaic' *H. sapiens* and thence into 'modern' *H. sapiens*, then the earliest examples of *H. sapiens* should show *erectus*-like characteristics. The fossil record should progressively document the appearance of 'modern' features. Indeed, we might even expect there to be fossils with intermediate characteristics.

Table 1 Comparison of morphological traits, chosen on the basis of ability to differentiate hominid taxa, among four key specimens discussed in the text

	<i>A. africanus</i>	Intermediate	<i>H. habilis</i>	Intermediate	<i>H. erectus</i>	Intermediate	<i>H. sapiens</i>
Cranial capacity			*†	‡		§	
Naso-alveolar clivus	*			†			
Incisive alveolar margin	*		‡		†		
Canine juga		*	‡		†		
Subnasal prognathism		*					
Midfacial prognathism		*		†	‡		
Size of temporal fossa		*	†		‡		
Angle of zygomatic process of temporal			*				
Bizygomatic breadth relative to prosthion-nasion height		*		†‡			
Hafting of zygomatic process of maxillary				†			
Frontal plane concavity of nasals			*†‡				
Frontal plane concavity of lateral orbital rim			*†				
Frontal angle			*†	‡		§	
Cranial length relative to biporionic width		*	†				
Facial height relative to cranial length		*					
Thickness of parietal and temporal			†		‡		
Thickness of occipital					§		
Anterior protrusion of supraorbital torus				†‡			
Lateral thickening of supraorbital torus			‡		§		
Retraction of lateral supraorbital torus							§
Midline depression of supraorbital torus							§
Deep post-toral sulcus				†			
Post-orbital constriction				†			
Anterior temporal line robusticity					†‡		
Parasagittal flattening of frontals/sagittal keel			†	‡		§	
Supramastoid crest larger than suprasmatal crest			†‡				
Frontal process of zygomatic angled anteriorly downwards			†				
Occipital angle				†	§		
Torus occipitalis				‡		§	
Torus angularis					‡		
Low parietal angle							§
Euryon at torus angularis					‡		
Deep and wide zygomatics					‡		
Mesio-distal diameter of molar row					‡		
Upper facial breadth relative to orbital height						§	
Supraorbital and maxillary pneumatization							§
Cranial vault height						§	
Relatively large diagonal dimensions of parietal							§
Temporal squama short and arched							§
Axis of tympanic plate							§
Nuchal area					§		
Lambda-inion and inion-opisthion chords sub-equal						§	
Broadness of cranial base						§	
Palate size						§	

This chart shows the mosaic pattern of evolutionary change in hominids, the clear directionality to be seen in a number of morphological traits, such as cranial capacity, and the fact that there are hominid fossils showing morphology intermediate between accepted hominid species.

* KMN-ER 1470, classified as *H. habilis*, shows nine traits which are either shared with *A. africanus* (Sts5, AL 288-1, MLD 37/38) or intermediate between this taxon and other *H. habilis* (OH 7, OH 13, OH 24, L 894-1, SK 847).

† KMN-ER 1813, a later *H. habilis*, shows 11 traits characteristic of *H. erectus* or intermediate between *H. erectus* and *H. habilis*.

‡ KMN-ER 3733, classified as early *H. erectus*, shows 11 traits shared with *H. habilis* or intermediate between this taxon and other (Asian) *H. erectus*.

§ The Petralona cranium classified here as an early *H. sapiens* shows 13 traits either shared with *H. erectus* or intermediate in morphology.

The European Middle Pleistocene record does provide us with such examples. The fossils from Mauer, Arago, Vertésszöllös, Petralona and Bilzingsleben have been regarded by various workers as belonging to either *H. erectus* or *H. sapiens*^{33,43,45,46,83-85}. The most complete of the specimens is the Petralona cranium, apparently associated with a Biharian (Cromerian) fauna which would place it as broadly contemporaneous with the Mauer, Vertésszöllös and perhaps Choukoutien fossils, and earlier than the Bilzingsleben, Swanscombe and Steinheim specimens⁸⁴. If phyletic gradualism operated to transform *H. erectus* into *H. sapiens*, a specimen from the middle part of the Middle Pleistocene would be expected to show intermediate or mosaic characteristics when compared with the two species. Table 1 shows that the Petralona cranium displays characteristics typical of each species (if we can accept that Neandertals do in fact belong in the species *H. sapiens*). In features associated with cranial expansion and dental reduction, it is apparently intermediate. All the earlier Middle Pleistocene European specimens may belong in the species *H. sapiens*, but placing them within *H. erectus* would not alter their evolutionary significance. They differ as much from early *H. erectus* fossils as they do from late *H. sapiens* specimens.

Vlček⁸⁵ has claimed that during the Holsteinian of Europe, *H. erectus* (as at Bilzingsleben) co-existed with *H. sapiens* (as at Swanscombe and Steinheim). Apart from the important ques-

tion of morphological variability to be expected in the Middle Pleistocene, correlations in Europe are now so uncertain that 'Holsteinian' faunas may, in fact, span two or more distinct interglacials covering a period of some 200,000 yr (ref. 86), and a recent study of the Bilzingsleben site suggests that it dates from the penultimate interglacial in Europe rather than the Holsteinian⁸⁷. Similarly, Swanscombe may be separated by a considerable period of time from other sites in the 'Holsteinian' complex. Certainly the morphology of the Bilzingsleben specimens suggests that present concepts of variation within the species *H. erectus* and *H. sapiens* need to be re-examined⁸⁸⁻⁹⁰.

In the fossil record of the later Pleistocene of Europe we meet the continuing problem of the place of 'Neandertals' in human evolution^{91,144,155}. Stringer⁴⁶ has discussed the European evidence for a gradual emergence of a Neandertal morphology during the later Middle Pleistocene and early Late Pleistocene. Fossils such as Swanscombe and Fontéchevade, previously regarded as part of a 'pre-sapiens' evolutionary lineage separate from that of the Neandertals, are probably part of an early Neandertal lineage. However, European Neandertals may not be the best candidates for the ancestors of anatomically modern *H. sapiens* in Europe during the middle part of the last glaciation^{46,47}, notwithstanding much support for the opposite view that there is an evolutionary continuity between these European populations^{45,81,83}. Until some of the critical specimens in this debate are conclusively dated and the functional significance of

some of the relevant morphological differences better understood, resolution of this problem may lie in the analysis of character states present in Neandertals and other contemporaneous populations^{48,90-95}.

Thus the evolution of anatomically modern man can at present support either the model of evolutionary gradualism or that of punctuated equilibrium, although the present evidence may favour discontinuity between Neandertal and 'modern' populations in Europe and Southwest Asia. Had Europe been a relatively peripheral area, gradualism in other areas could have led to the evolution of populations of anatomically modern *H. sapiens* whose genes could have infiltrated into Europe relatively rapidly, producing an apparent example of punctuated equilibrium in the European record.

Punctuated evolutionary change would imply a high variance in the durations or lifetimes of hominid taxa, as the appearance of new taxa is supposedly a function of a rapid and random event. If the taxa *A. africanus* extended from 3.2 to 2.2 Myr (1.0 Myr), *H. habilis* from 2.2 to 1.6 Myr (0.6 Myr), *H. erectus* from 1.6 to 0.6 Myr (1.0 Myr), and *H. sapiens* appeared at 0.6 Myr, then change does not seem to be dissimilar from one species to the next. If Laetoli *A. afarensis* at 3.7 Myr is accepted as a forerunner of *A. africanus*, this species further supports this pattern, with a known species lifetime of 0.5 Myr. For these five taxa, whatever their phylogenetic relationship, there is a mean duration of 0.74 ± 0.24 Myr, indicating similar species lifetimes, as may be expected in a gradualistic model. For an alternative interpretation see Stanley⁹⁶.

Possible cases of punctuated equilibrium in the hominid fossil record

The geologically sudden appearance of robust australopithecines in South and East Africa between 2.1 and 2.0 Myr seems to represent an obvious example of punctuation. The fact that their morphology appears so specialized supports this. However, on closer examination the robust lineage reveals a distinctly gradual trend of evolution. It is the highly specialized and hence accountable morphology of the 'final product' that enables one to recognize the direction of the evolutionary trend and to define the position of each fossil within the sequence.

Rak⁹⁷ explains this trend as related to the forward movement of the origin of the masticatory muscles away from the fulcrum (the articular eminence), on the one hand, and the tucking in of the dental arcade, on the other. *Australopithecus boisei* is the most evolved species of the sequence. However, some of the *A. boisei* specimens, whether studied through actual measurements or through observations of their topography, are found to fall within the range of the less specialized species, *A. robustus*. The direction of evolution of the masticatory system is also detected in the known sample of *A. robustus*. The topography observed on many fragments (mainly the infraorbital region) again indicates the existence of a morphological sequence. *A. robustus* and *A. boisei* are ordered in this sequence, although significant differences exist to justify their recognition as distinct taxa. The claims¹ that 'there is no direct evidence for gradualism within any hominid taxon' and 'each species disappears looking much as it did at its origin' probably result from a familiarity with only the most well known specimens.

The known sample of *A. robustus* is probably contemporary with at least part of the time span of *A. boisei*. Morphologically, however, *A. robustus* would be expected to be an intermediate stage between the more general australopithecines and the more specialized, hyper-robust *A. boisei*. One hypothesis to explain the relationship between *A. robustus* and *A. boisei* is that the former represents a relic population that continued to exist in South Africa, representing the prototype from which *A. boisei* evolved.

Some of the specimens which make up the morphological range of species of *Australopithecus*, that is *A. africanus*, that existed 0.5 Myr earlier, resemble *A. robustus* very closely in topographical features of the face. The fairly complete fossil Sts

71 can be considered representative of the robust end of the morphological range, although fragments and isolated teeth of other specimens hint at the possibility that even more robust individuals existed. Specimens from the other end of the range, such as Sts 52 and TM 1512, exhibit a quite generalized primitive morphology. Rak⁹⁷ suggests that *A. africanus* may have a greater morphological spread than the sample of *A. robustus*, not because the former is represented by a larger sample (it is not), but because a longer time span is covered by its sample.

Robust australopithecines probably evolved from earlier, more gracile hominids similar in morphology to *A. africanus*. Whether the known fossils of this taxon represent the actual ancestral population of later robust australopithecines is debatable (Fig. 2) but *A. africanus* is clearly morphologically closer to a common australopithecine ancestor than are robust australopithecines. Continuities to be seen from gracile to robust australopithecines therefore do not support a punctuational view of australopithecine evolution.

Other parts of the hominid fossil record suggest that stasis may have predominated over evolutionary change in certain peripheral areas. The Ngandong crania are supposedly Late Pleistocene in age⁷⁴ but show fundamental resemblances to much earlier *H. erectus* specimens^{46,98}. As with the Broken Hill specimen⁹⁹, this apparent Late Pleistocene anomaly may eventually be removed by evidence that the Ngandong fossils are, in fact, of Middle Pleistocene age; but at the moment these fossils provide one of the strongest examples of relative stasis in a peripheral and relict human population.

Evidence from genetic studies

Genetic study of current populations is a major focus of attempts to elucidate patterns and mechanisms of speciation¹⁰⁰⁻¹⁰³. The traditional view—that there are large genetic changes, or 'a

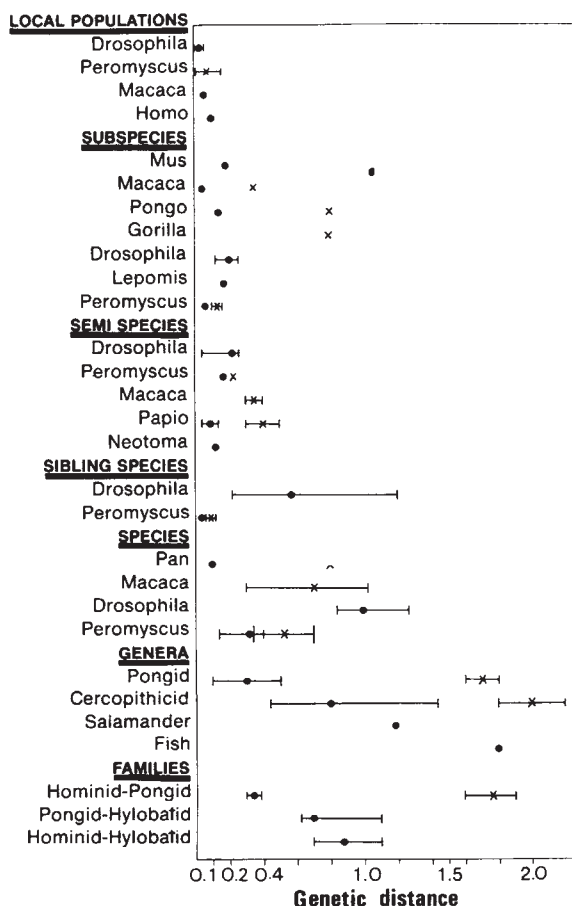


Fig. 5 Range of genetic distances (bottom axis) for the varying taxonomic levels as referenced in Table 2. ×, Fast loci; •, typical mix of loci.

Table 2 Genetic distance values for taxa compared at different 'levels' of speciation

Taxon	Genetic distance				
	<i>D</i> typical			<i>D</i> fast	
	<i>Drosophila</i>	Vertebrates	Primates	<i>Peromyscus</i>	Primates
Populations	0.02 ± 0.012	0.028 ± 0.02	0.011 ± 0.012	0.066 ± 0.06	0.30 ± 0.235
Sub-species	0.186 ± 0.057	0.163 ± 0.02	0.084 ± 0.048	0.139 ± 0.009	0.477 ± 0.239
Semi-species	0.226	0.168 ± 0.01	0.163 ± 0.14	0.224	0.4 ± 0.086
Species	0.451 ± 0.329	0.319 ± 0.194	0.318 ± 0.233	0.305 ± 0.257	0.67 ± 0.276
Genera	—	0.706 ± 0.319	0.631 ± 0.31	—	1.36 ± 0.512

The term semi-species is defined by Mayr¹⁰² as species that were recently conspecific and subsequently speciated through geographical isolation and remain mostly allopatric. Hybridization does occur to differing extents (refs 7, 101, 115, 123, 124, 126, 127, 134–138, and J. E. C. and V. M. Sarich, unpublished observations).

genetic revolution', often accompanying speciation events—would be supported if the process occurred in small, peripheral or isolated populations^{104–109}. Various papers have reviewed the extent of genetic variation in natural populations^{110,111} and genomic distances can be quantified between taxa^{112,113}. Gould and Eldredge¹ have cited structural gene studies of allozymic differences between taxa of differing levels as the major criterion of their model. Ayala¹¹⁴ and Avise¹¹⁵ (Table 2) have indeed observed a gap between genetic distances as measured at both the local population level and at the level of sub- and semi-species but Lewontin¹¹⁶ and White¹⁰¹ find little genetic distance accumulating during speciation. Many species are less different genetically than populations within the same species, and morphological diversity may not correlate with genic divergence^{101,117,118}.

The apparent gap between populations and measures between sub- and semi-species is due to a differential weighing of loci with different function^{119,120} and different rates of evolution^{7,121}. Work on primates^{121–123} and on rodents¹²⁴ demonstrate that this gap disappears when fast-evolving loci are included in the study (Table 2, Fig. 5). In fact allozymic differences between taxa may be highly time-dependent and useful for divergence date estimates^{6,7,121,122}, an observation not compatible with suggestions of punctuation or genetic revolution.

Hence there is no obvious correlation between the degree of genetic differentiation and speciation; the development of reproductive isolation, by whatever mode, may be accompanied by little, some, or substantial allozymic change.

Electrophoresis of gene products may well be too insensitive a method to detect the genetic changes accompanying speciation. Nucleic acid hybridization shows that species differ by quantitatively small values of base-pair difference, much less than 1% in unique sequence DNA. Sub- and semi-species differ by even less¹²⁵.

So what is the genetic basis of speciation? Microgenetic changes may result cumulatively in reproductive isolation. Single gene mutations at structural loci may have a profound effect on the phenotype and correspondingly to speciation events. Alternatively, not all genes may be involved in a genomic reorganization. A model of genetic 'transilience'¹¹⁸ in which only a few polygenic systems each with major and minor genes undergo change is also possible. Thus a change in only a small fraction of the genes could contribute to a genomic revolution.

Also, true revolution may be found at the chromosomal level. Chromosomal rates of evolution seem to correlate with the degree of morphological evolution and rates of speciation¹⁰⁷. Such change would be macrogenetic and would best be sought at the level of satellite DNA, quantity of DNA per locus, chromosomal rearrangements and regulatory loci mutations¹⁰¹.

Conclusions

We should not be surprised to find specific instances of explosive and gradual evolution at both the molecular and organismal level. The questions at hand are whether punctuation or saltation is the predominant mode of evolution as Gould and

Eldredge assert¹, and whether human evolution is a case which confirms the general model of punctuated equilibrium at either the genetic or morphological level.

The specific course of genomic evolution along the hominid lineage can be conjectured. Since the time of divergence, humans and chimps have come to differ by about 1% at each gene¹²⁶, a value characteristic of differences generally found between sibling species^{122,126,127}. On the other hand, humans and chimps differ by some nine pericentric inversions¹²⁸. Fixation of such inversions is facilitated by low effective breeding size of populations or inbreeding^{101,129}. Conditions for fixation of such inversions would be optimal during speciation by bottlenecks. Thus, it is highly probable that a few bottlenecks in effective breeding size, some resulting in speciation, without apparent major effects on the phenotype may have occurred along the hominid lineage.

Perhaps the most interesting area of evolution—the origins of the human lineage—can only be cautiously dealt with due to incomplete evidence. It may be the most likely period of time when rapid or explosive evolution may have occurred. The earliest undoubted hominids in the fossil record, from Hadar and Laetoli⁹, are quite primitive and date to somewhat less than 4.0 Myr. The molecular clock suggests a hominid-pongid divergence somewhat before this^{6,7}. Thus, the hominid transition could have been characterized by extremely rapid morphological change (the evolution of bipedality), and by a period of low population numbers, or bottlenecks, with most of chromosomal evolution, genic reorganization, or allelic substitutions occurring in rapid sequence. It is here that the evidence fails us in being particularly incomplete, only partially suggestive and open to many different interpretations^{130,131}, and it may be that as more evidence becomes available even this possible case of punctuation may be like the cheshire cat and disappear until only its smile is left.

In summary, the evidence that we, unlike some others^{96,132,133}, have adduced from the fossil record does not support the model of punctuated equilibrium for hominid evolution. Apparent cases of punctuated equilibrium in the fossil record of hominids are due to one of three causes. Specimens are certainly misdated, as in the case of KNM-ER 1470, possibly or probably misdated, such as the Ngandong specimens, or open to very different morphological interpretations, for example, regarding KNM-ER 1813 as a gracile australopithecine versus a member of the genus *Homo*. A review of the morphology of hominid fossils with their most probable dates strongly suggests a closer approximation to a gradualistic model. There is demonstrable change within hominid taxa through time as well as continuities from one species to its supposed descendent species. There is little or no support for the belief that the hominid fossil record is complete. Thus gaps in the record are most likely artefacts of the processes of fossilization and discovery and not true palaeobiological discontinuities. Further assessment of the model of punctuated equilibrium must await a more rigorous definition of such parameters as the temporal difference between punctuation and fast gradualism and the degree of morphological change considered to constitute either a gap or stasis. If a finer, continuous analysis were possible, it

would probably reveal that gradualism is, itself, an average of periods of horotely (fast change) and bradytely (slow change). Evolution of the Hominidae is still most reasonably interpreted by a model of phyletic gradualism with varying rates.

We thank M. Billig, L. Brunner, R. Happel, K. Janszen, R. Dorit, E. Meikle and S. L. Washburn for helpful discussions and for comments on previous drafts of the manuscripts, F. C.

Howell and A. Wilson for access to laboratory space, and F. C. Howell for use of the original drawings in Fig. 3. We also thank J. Ogden and W. Powell for their illustrations. Grants from Wenner Gren Foundation, NSF, L. S. B. Leakey Foundation, Milton Fund of Harvard University and Research Challenge Fund of New York University supported aspects of the background research for this paper.

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ARTICLES

Depth of geological contrast across the West African craton margin

J. C. Briden, D. N. Whitcombe, G. W. Stuart & J. D. Fairhead

Department of Earth Sciences, The University of Leeds, Leeds LS2 9JT, UK

C. Dorbath & L. Dorbath

Office de la Recherche Scientifique et Technique Outre-Mer, 24 Rue Bayard, 75008 Paris, France

Anomalies in teleseismic arrivals at stations astride the West African Craton margin in Senegal are large and systematic for rays which have passed beneath the craton margin. Lateral variation in seismic velocity structure beneath the margin persists to several hundred kilometres depth. The major relative delay time and slowness–azimuth anomalies fit a simple model of a steep boundary in the mantle aligned with the major gravity anomaly associated with the craton margin. The mantle beneath the Mauritanide orogenic belt has lower P-velocity than the adjacent craton between ~80 and 220 km, but higher average velocity both above and below that depth range.

If mantle structure could simply be categorized as sub-continental and sub-oceanic, there would be no consensus over whether lateral differences persist to depths greater than ~220 km (refs 1–5). Seismological and other evidence indicate a more complex regional characterization of mantle structure. Significant lateral differences occur down to at least 500 km in the vicinity of many active subduction zones^{6,7}. Theory of formation of orogenic belts at convergent plate margins requires the persistence of subduction, and hence of lateral variations to great depths, throughout the period of orogenesis. Surface wave data from Tertiary⁸ and older orogenic belts⁹ suggest that shear wave velocity structure beneath such belts differs from that beneath both ancient cratons and modern oceans. Global and regional teleseismic delay times^{10,11} and heat flow^{12,13} have been taken to indicate lithospheric thicknesses >200 km in places. Thus differences which arose at the time of orogenesis have persisted for hundreds of millions of years after the cessation of orogenic activity so that determination of present-day seismic structure of ancient orogenic belts may clarify the geodynamics of orogenesis.

We report here preliminary results for temporary teleseismic arrays laid out across the junction of a Precambrian craton with a Phanerozoic orogenic belt to detect lateral differences in mantle seismic velocity structure and to determine whether a boundary between these two regions is detectable to mantle depths.

Location and design of field experiment

Figure 1 shows the site of the experiment in Eastern Senegal, where the western margin of the West African craton (~2,000 Myr and older) abuts the Mauritanide orogenic belt of late Palaeozoic age (~250 Myr).

There are differences of up to 1.0 s in teleseismic delay times between seismic stations on the craton (Kedougou, Fig. 1) and on the orogenic belt¹⁴. The major long-wavelength Bouguer gravity anomaly¹⁵ parallel to the craton margin is characteristic of many craton margins elsewhere^{16,17}. These data suggest that lateral differences across the margin may persist at least into the uppermost mantle.

Two short period vertical (SPz) seismic arrays, each of 20 km aperture, were operated for 6 months: the L-shaped nine station Kedougou (Ked) array on the craton and the cross-shaped eight station Missira (Mis) array centred 180 km to the north-west on the orogenic belt provided travel-time and slowness-data necessary for three-dimensional interpretation of the structure beneath and between them. A mobile linear array of three or four SPz seismometers was placed for 1 month in each of five

successive positions A–E (Fig. 1) to provide records at 10-km spacing along the whole profile between the Ked and Mis arrays. A multichannel analogue tape recorder at the central station of each array recorded an adjacent three component set of short-period seismometers and a single vertical long-period seismometer together with the radio-telemetered signals from the outstations of each array. The internal time base of each recorder was made absolute by recording time signals from BBC World Service transmissions.

All recorded events were digitized at 50 samples per s, and relative arrival times were picked by waveform matching producing overall accuracy of better than 0.05 s. Epicentral

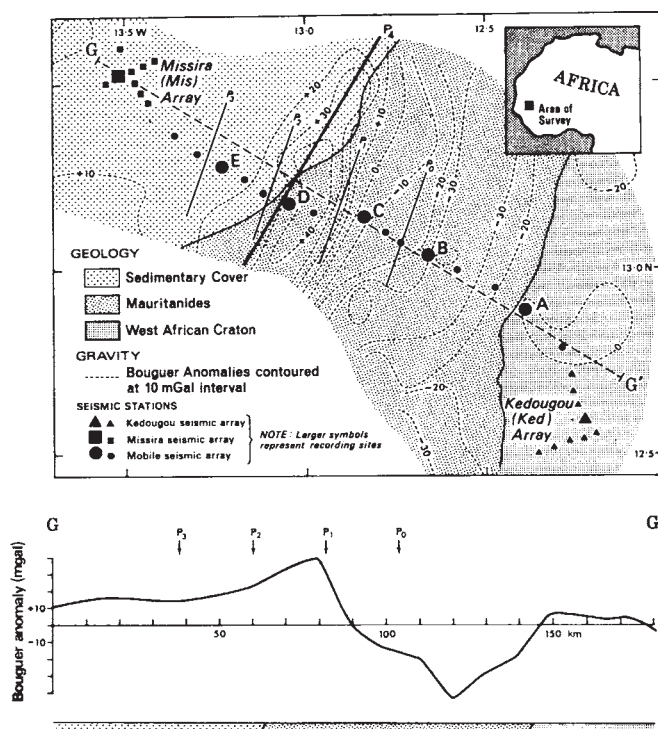


Fig. 1 Part of Senegal, showing the seismic arrays, the Bouguer gravity contours¹⁵, and the Bouguer anomaly profile along the line GG'. P₀ to P₄ are examined positions of a postulated boundary separating cratonic and orogenic velocity structures.

information was taken from USGS PDE bulletins and predicted arrival times were computed utilizing the programme GEDESS¹⁸ with the Jeffreys-Bullen travel-time tables¹⁹. To date 550 seismic events have been identified on the analogue tapes, of which 330 originated in the teleseismic P window, 120 were core phases and the remainder were of local origin. This report considers a subset of the SPz records at the main Ked and Mis arrays, of teleseismic events for which delay times and apparent velocities have been determined, together with some delay time data from the mobile array.

Results

The delay time differences between the central stations of the Mis and Ked arrays are plotted as a function of epicentral distance and azimuth in Fig. 2a. The main features are: rays into the Mis array on the orogenic belt from almost all azimuths and teleseismic distances are slower (positive delays) relative to those into the Ked array on the craton. However, the magnitude of the delays is sensitive to distance and azimuth; between azimuths of $\sim 40^\circ$ and 70° there is a rapid decrease in relative delay time from +0.8 to -0.2 s. The maximum relative delays (+0.8 s) occur for events from the north-east. For westerly azimuths, the delay times decrease with increasing distance from 0.7 s at $\Delta = 60^\circ$ to 0.3 s at $\Delta = 80^\circ$. The relative delays have also been determined for a set of PKIKP core phases in the distance ranges $174^\circ < \Delta < 178^\circ$ and show a similar constant delay of +0.3 s.

Variations of relative delay time along the whole profile are shown in Fig. 3. Each nuclear test site (Fig. 3a-c) can be regarded as a single repeated source. The Kazakh data (Fig.

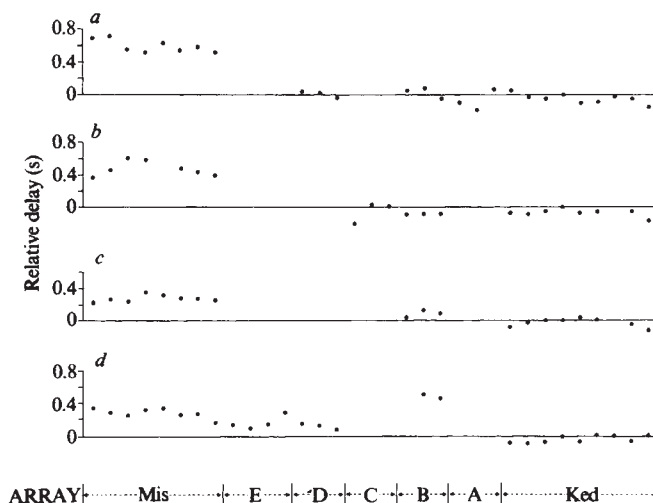


Fig. 3 Teleseismic delay time profiles for underground nuclear tests at *a*, East Kazakhstan $\Delta = 80^\circ$, $\phi = 41^\circ$; *b*, West Kazakhstan $\Delta = 61^\circ$, $\phi = 42^\circ$; *c*, Southern Nevada $\Delta = 92^\circ$, $\phi = 309^\circ$; and *d* for PKIKP phases in the range $174\text{--}178^\circ$.

3a, b) show a sharp westerly increase in relative delay within array E, ~ 80 km west of the outcropping margin. The PKIKP phases (Fig. 3d) have all travelled by similar paths through the receiver structure; they do not show the same pattern of relative delay, though they do show an anomalous (though poorly defined) delay within array B.

Slowness-azimuth anomalies at the Ked array (Fig. 4b) are small and non-systematic from all azimuths except the west; for westerly paths (for rays which have passed beneath the craton-orogenic margin) slownesses are anomalously high and seem to have been refracted across a structure striking NNE. At the Mis array the dominant feature (Fig. 4a) is southerly slowness-azimuth anomalies which are thought to be due to a northerly dipping sedimentary-basement interface.

When the effect of this interface is 'stripped-off' rays from only two sectors appear anomalous (Fig. 4c). Northeasterly arrivals, which have traversed beneath the craton margin, seem to have been perturbed by a NNW-striking structure—slowness being anomalously low for the steepest arrivals but anomalously high for arrivals at larger incidence. Anomalies which persist for azimuths close to 280° could be due to lateral variation in the deep structure of the orogenic belt.

Two-dimensional velocity models

Because the regional strike of the gravity anomaly, the craton margin and the orogenic structure are generally constant and the slowness anomalies follow their local variation, we can treat the seismic data two-dimensionally on a regional scale. In our velocity models we adopt a compromise strike of our models (P_0 – P_3) between the trends of the segments of the line P_4 in Fig. 5.

Models of crustal structure which would produce the observed anomalies all have unacceptable features, notably large lateral velocity contrasts and/or Moho topography which are incompatible with the observed gravity field across the arrays. Hence velocity differences must extend into the upper mantle. The craton has predominantly the higher velocity structure because vertically travelling core phases through the sub-orogenic structure are delayed by 0.3 s. We treat the structure beneath the Mis and Ked arrays as essentially horizontally layered because these arrays are clear of the craton margin and its associated gravity anomaly, and except for ray paths which pass beneath the craton margin, the slowness-azimuth anomaly patterns do not suggest inclined layering at depths²⁰. For such models the azimuth-distance dependence of relative delay times and the drastic change in direction of the slowness-azimuth anomaly vectors for northeasterly arrivals into Mis, both require

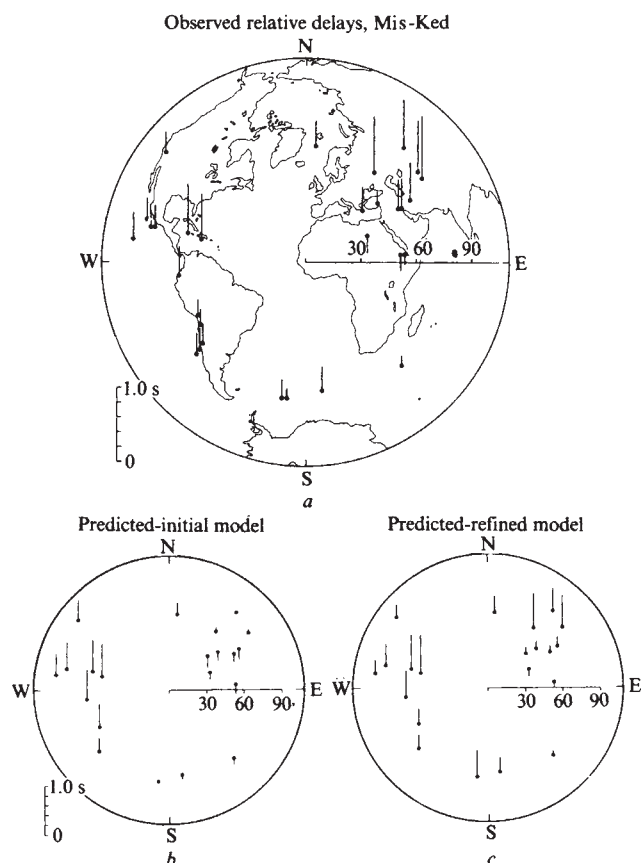


Fig. 2 Teleseismic delay time differences Mis-Ked between the Missira and Kedougou arrays as a function of the position of the source; *a*, observed; *b*, predicted by ray tracing through the starting model of Fig. 6a in which the craton boundary is at position P_3 and vertical; *c*, predicted by ray tracing through the refined model of Fig. 6b in which the craton boundary is at position P_2 and dips at 85° to the west.

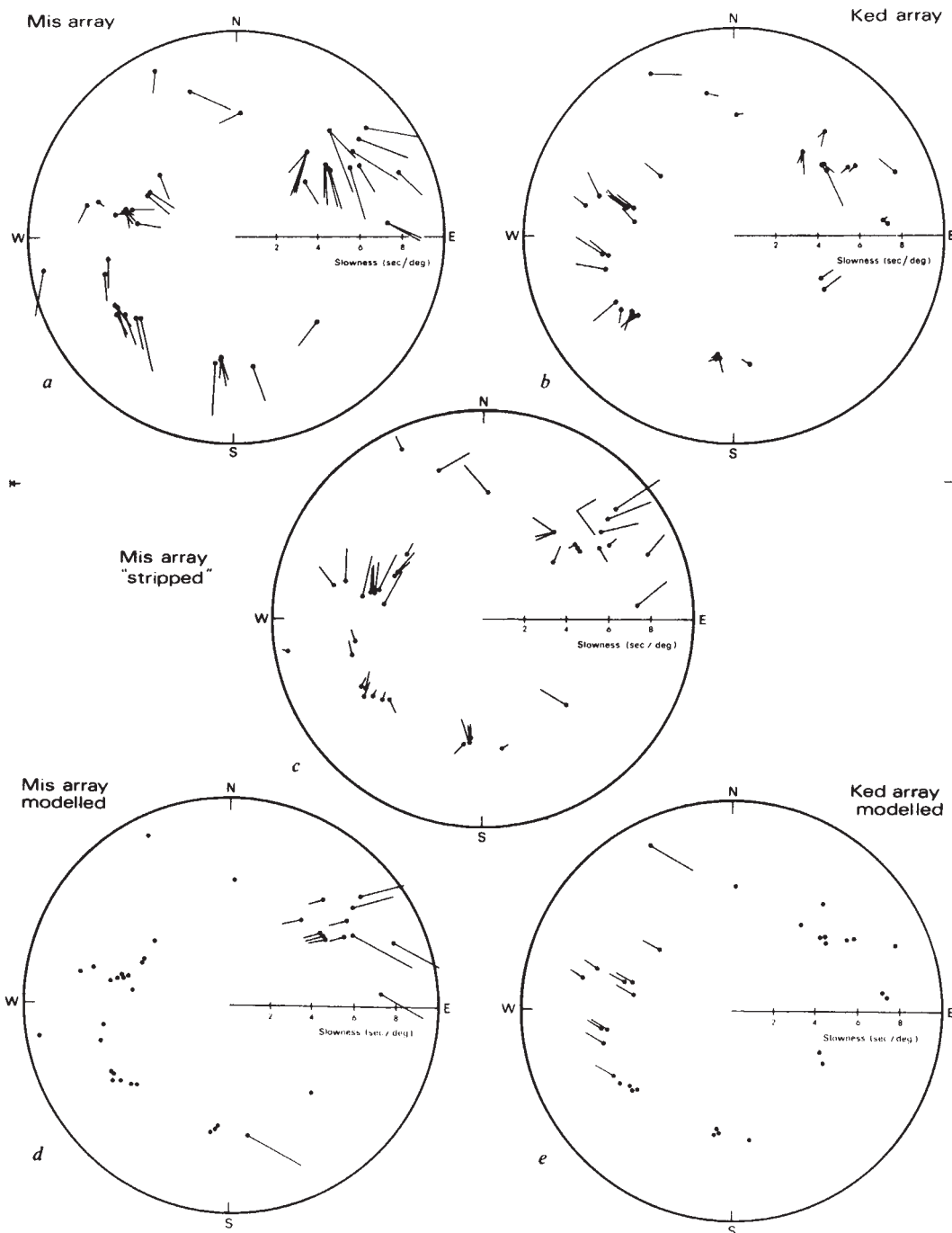


Fig. 4 Slowness anomalies for *a*, Missira; and *b*, Kedougou arrays²⁰. ●, Expected slowness. Anomalies generated at a single interface are orthogonal to the strike of that interface. In *c* the effect of basement topography dipping at 348° azimuth is stripped-off by subtraction of a vector 1.7 s deg⁻¹ in that direction. In *d* and *e* the observations in *c* and *b* are modelled by ray tracing²¹ through the velocity structure of Fig. 6c in the position indicated in Fig. 5.

that between certain depth ranges the orogenic belt has the higher seismic velocity structure. The abrupt change in relative delay times along the profile (Fig. 3) and its marked variation with azimuth (Fig. 2a) require that the transition between the sub-cratonic and sub-orogenic velocity structure is both sharp and steep even at mantle depths.

Two-layer models which account for these anomalous features cannot also account for the negative relative delay times for easterly ray paths (Fig. 2a); these can be accommodated if the orogenic crust is thinner and/or has higher velocity than the cratonic crust. Thus to explain all the main features of the data, we need a model with a minimum of three 'layers' in the crust and upper mantle in which the laterally uniform sub-craton and sub-orogen velocity structures are separated by a near vertical boundary; the top and bottom layers have the higher average velocities beneath the orogenic belt and the middle layer has the higher velocity beneath the craton.

Each layer *i* delays an arrival travelling through the sub-orogenic structure by a time *a_i* relative to an arrival travelling through the same layer in the sub-cratonic structure: thus *a₂* will be positive while *a₁* and *a₃* are negative. Vertically-travelling core phases travel entirely in either sub-orogenic and sub-cratonic velocity structures, so that *a₁ + a₂ + a₃* = 0.3 s. The same relative delay terms *a_i* apply to inclined teleseismic paths, errors due to non-verticality being negligible. The relative delays for these inclined paths will depend on the amount of mixing of the rays through the two structures. For example, the high relative delay of +0.8 s from the north-east is interpreted as being due to rays into Mis crossing the model boundary near the bottom of the middle layer: hence *a₁ + a₂* = 0.8 s. Similarly, negative relative delays for rays from the east which cross the boundary within the top layer, make *a₁* ≈ -0.2 s. Thus *a₂* = 1.0 s and *a₃* = -0.5 s.

If *h_i* is the thickness of the *i*th layer then *a_i* is related to the

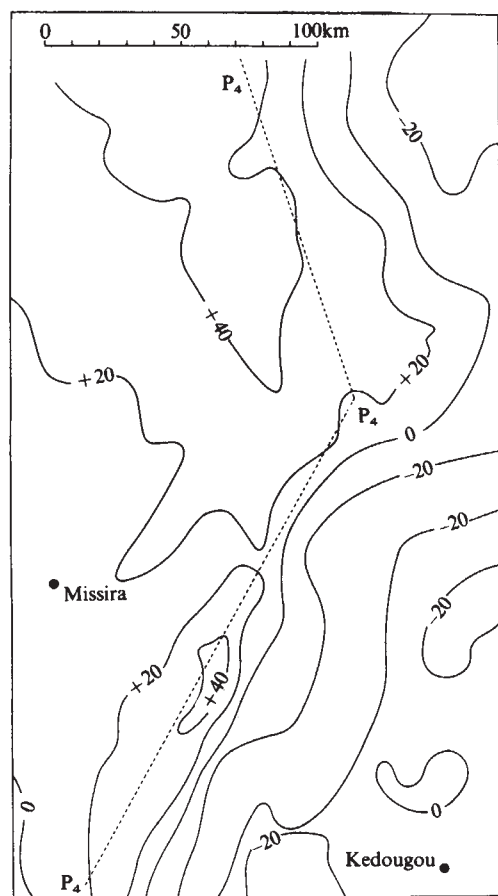


Fig. 5 The position of the seismic boundary P_4 , inferred from the slowness models (Fig. 4d, e) superimposed on the Bouguer anomaly map¹⁵.

average velocities V_{oi} and V_{ci} within that layer beneath the craton and orogen respectively by

$$a_i = h_i \left(\frac{1}{V_{oi}} - \frac{1}{V_{ci}} \right)$$

hence

$$\frac{\delta V_i}{V_i} \approx \frac{V_i a_i}{h_i}$$

where V_i is the average velocity within layer i and $\delta V_i = V_{ci} - V_{oi}$.

Interpretation of the data requires the thickness of the layers and the position of the boundary to be determined. To satisfy the slowness anomalies and delay time variations from westerly azimuths, rays to Ked from the west must cross the boundary across a negative velocity contrast (within the bottom layer). The largest positive delay times for rays into Mis from easterly azimuths must cross the boundary near the base of layer 2. These two constraints can be met if the boundary at depth occurs west of its outcropping position—a conclusion supported by the nuclear explosion profile data (Fig. 3). More easterly estimates of the position of this boundary lead to contradictory depth estimates for westerly and northeasterly ray-path data. With the boundary at P_3 (Fig. 1) the thickness of the top layer is estimated at 80 km to account for the ray paths which show a relative delay of -0.2 s, assuming that they cross the boundary at the base of layer 1. Arrivals at Ked from the west, with anomalously large slowness, cross such a boundary in the depth range 220–390 km while those to Mis with relative delays of 0.8 s cross the boundary at ~ 240 km. Hence the base of layer 2 is taken at 230 km and that of layer 3 at 400 km for our starting model. This definition of model layer thicknesses h_i constrains the velocity

contrasts in each layer to be -1.7% , $+5.4\%$ and -2.8% respectively.

This starting model (Fig. 6a) may now be refined to reconcile all the relative delay time data and to allow for refraction across the boundary, using a 'shooting' ray tracing technique²¹. The fit of predicted and observed delay times for arrivals from the east is further improved by dividing the top layer into two (Fig. 6b). An inadequacy of the starting model is that signals from East Kazakhstan would be internally reflected at the boundary before reaching Mis. This problem would not exist with a lower velocity contrast in the bottom layer, or if the strike of the boundary were bent as in Fig. 5, or if the boundary were inclined at a small angle to the vertical so that it is dipping beneath the orogen. The teleseismic delay times are sensitive to the inclination of the boundary, as rays are travelling steeply through the model. It is difficult to satisfy the large relative delays from the north-east with the smaller relative delays from the west unless the dip of the boundary deviates from the vertical. The refined model (Fig. 6b), which has the surface expression of the boundary at P_2 , and the dip of the contact as 85° towards the west, displays the main features of the observed relative delay time pattern: compare Fig. 2c with Fig. 2a.

To investigate further the interdependence of model parameters, the relative delay times have been directly inverted by a modification of the least squares technique of Aki *et al.*²². This modification divides each layer of the model into two to simulate sub-cratonic and sub-orogenic structure. The block boundary, which need not be vertical, thus simulates the actual lateral velocity discontinuity within each layer. The travel time of each ray through each block is calculated using three-dimensional ray tracing²¹. This inversion method mimics the tectonic regionalizations common for surface wave dispersion studies²³. The trial models allowed for velocity differences down to 400 km, either side of a vertical or near vertical boundary in positions P_0 , P_1 , P_2 and P_3 (Fig. 1).

The lowest solution variance occurred with the boundary at a position P_2 , and dipping beneath the orogenic belt between 85° and 80° . This solution accommodated $\sim 80\%$ of the observed data variance. Ray tracing through this inversion model yields predicted delay times similar to those of Fig. 2c. The most consistent feature of all the solutions with variance close to the minimum is the region in which the sub-cratonic structure has the higher velocity: this occurs at a depth 80–200 km if the boundary is at the preferred position P_2 consistent with the

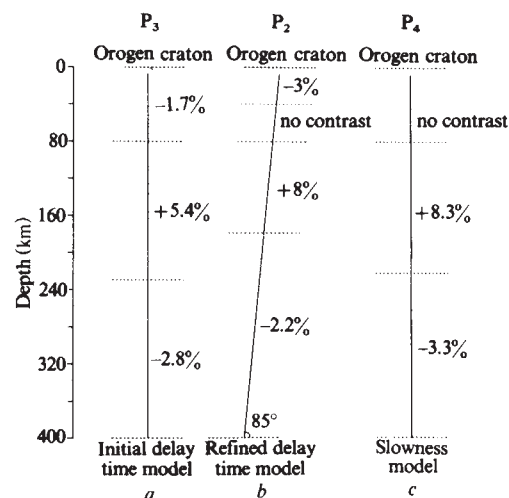


Fig. 6 Velocity contrast models obtained by forward modelling using three-dimensional ray tracing. For all these models the boundary between the craton and orogenic velocity structure is assumed to be planar and positioned at P_2 , P_3 or P_4 (see Fig. 1). The dip of the boundary is represented to true scale. The contrasts in the slowness model c must be greater to explain the magnitude of the observed slowness anomalies in Fig. 4.

deductions from forward ray tracing. If the boundary were only 20 km to the east (P_1) the equivalent layer would be displaced downwards by 40 km; a comparable upward displacement of this layer would be inferred if the boundary lay 20 km to the west (P_3).

All solutions tend to show higher average velocities beneath the orogenic belt below this layer down to 400 km (layer 3 in the ray tracing modelling). However, neither this 'layer' nor the detailed velocity structure within it is well defined, probably because the velocity perturbations are small and comparable with their standard errors.

Although the inversions do not define a top layer as clearly as the ray tracing approach they do suggest a faster velocity structure within the top 80 km beneath the orogenic belt as inferred from ray tracings. The velocity perturbations determined by the inversions are all smaller than the equivalent velocity perturbations obtained by ray tracing. Simulated data studies²⁸ have demonstrated that this inversion method can underestimate the magnitude of velocity anomalies.

Aspects of three-dimensional modelling

The slowness–azimuth anomaly patterns (Fig. 4b, c) require that their causative structure should not be precisely linear. A seismic boundary positioned along the line P_4 in Fig. 5 gives a 'best' fit to the data in conjunction with the velocity contrast model in Fig. 6c in that it reproduces the magnitude and direction of the slowness anomalies for westerly paths into Kedougou and the antiparallel slowness anomaly for the NNW event (Fig. 4e) and reproduces the size, orientation and (to some extent) azimuth dependence of the slowness anomalies from the north-east quadrant into Missira (Fig. 4d). Because the line P_4 was chosen on the basis of the seismic data, the concordance of its strike with that of the Bouguer anomaly (Fig. 5) is noteworthy. (The coincidence of its position in Fig. 5 is of less significance because position and dip of the model boundary and depths of the layers are to some extent mutually traded-off in the search for acceptable models.)

Although the teleseismic P and PKIKP data recorded by the end-arrays are fitted well by the models, the isolated observation of large relative delays for a PKIKP event recorded within subarray B (Fig. 3d) is not predicted, and the more complex model required must await more data, notably from the mobile array.

Tectonic interpretation of the seismic models

The contrast in background Bouguer anomaly between the craton and the orogenic belt (Fig. 1) is consistent with the cratonic crust being either thicker or having lower average density (and seismic velocity) than the orogenic crust, and hence is consistent with the uppermost part of the seismic model (Fig. 6).

The seismically-determined craton margin at depth lies ~80 km west of the outcropping margin, implying at least that amount of thrusting and/or depositional overlap of the rocks of the orogenic belt onto the craton. This intervening terrain is composed of a metamorphic belt (correlating with the linear positive Bouguer anomaly) and a zone of unmetamorphosed sediments and volcanics (correlating with the negative Bouguer anomaly, and pinching out with it in the north of Senegal); these correlations suggest that the causes of the linear Bouguer anomalies are within the overlap–overthrust rocks of the upper crust.

The major seismic velocity contrasts along the profile occur within the depth range ~80–200 km where the sub-cratonic structure is the faster by ~6%. This result is consistent with delay time inversion studies of lateral differences between older and younger orogenic regions in North America^{3,24} and in Europe²⁵ with the older structures having the higher velocities. The depth range 80–200 km also correlates well with the S-wave low velocity zone determined by the inversion of surface waves beneath aseismic continents²⁶. We thus prefer to ascribe the 6% P velocity contrast to a low velocity sub-orogenic

structure rather than to an anomalously high velocity sub-cratonic structure.

At depths ≥ 200 km the younger, sub-orogenic, structure has the higher velocity, although the contrast is not as marked (~3%). It may be argued that such a small contrast may be a random fluctuation in velocity, and not correlatable with the tectonics. However, studies in North America and Europe^{3,24,25} have revealed similar features: cratonic North America has 2–3% lower velocity in the depth range 250–400 km than both the Appalachians and Western Cordillera. Similarly the Precambrian Grenville Province has lower velocities than the Palaeozoic Appalachian Province for the depth range 200–350 km (ref. 24). Within Europe in the depth range 250–400 km the P-wave velocity beneath the Alps is up to 2% higher than beneath adjacent areas²⁵. Furthermore the interpretation of NORSAR travel-time data using the Herglotz–Wiechert inversion technique indicates that the Baltic craton has higher velocities than orogenic Europe down to 300 km, while in the depth range 300–420 km it has the lower velocities²⁷.

Because of the difficulties²⁹ of determining the depth of origin of teleseismic travel time anomalies, it is worth emphasizing that the principal grounds of our identification of deep lateral variations are: (1) the large epicentral dependence of relative delay times of ~1.0 s require lateral variations in upper mantle structure; (2) slowness anomaly vectors orthogonal to the local strike of the long wavelength gravity anomaly and craton margin imply that the seismic anomalies are inherently associated with that margin. Furthermore a reversal of the anomaly vectors at each array provides direct evidence for a velocity contrast reversal with depth. Such a model will accurately predict the major delay time anomalies.

If density contrasts at depth alternate then isostatic compensation depths may be much deeper (~400 km) than hitherto suspected. The gravity anomaly observed at surface may be regarded as the sum of several individual anomalies of alternate sign, generated in alternate depth zones: because these contributions will partially cancel each other out, the observed anomaly will not fully reflect the magnitude and extent of sub-surface contrasts. Relative teleseismic delay times for vertical paths to cratonic and orogenic stations show a similar effect if the boundary is near vertical: if we had taken delay times for vertical paths only or calculated a mean delay time for each station we would have inferred much less contrast and our models would not have extended to such great depths. It is the larger differential delay times from inclined ray paths which allow this deeper structure to be recognized.

It is beyond the scope of this article to speculate on the possible origin of a mantle boundary such as that inferred here, extending essentially vertically to 400 km into the mantle, but some preliminary points are worth noting. For example, where the lithospheric thickness exceeds ~300 km most sources of continental magmatism must be travelling with the lithospheric plate and must evolve with time, with or without replenishing mechanisms. If lithospheric thickness is variable, then the asthenosphere has considerable topography and variable thickness which would act as controls on plate motion. The near vertical attitude of a boundary within the upper mantle is not obviously compatible with an origin either at a compressional plate margin or an extensional margin; explanations may have to be sought in relation to transcurrent tectonics³⁰ (as has been suggested for the north-west margin of the West African craton on palaeomagnetic grounds³¹) or ideas about the deep thermal structure of plate margins may need revising.

This research has been funded by the Natural Environment Research Council, and Office de la Recherche Scientifique et Technique Outre-Mer. We thank ORSTOM, the Institute of Geological Sciences Edinburgh, and MOD(PE) Blacknest for assistance. IPG Contribution No. 513.

Received 4 February; accepted 14 May 1981.

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Purified λ regulatory protein cII positively activates promoters for lysogenic development

Hiroyuki Shimatake & Martin Rosenberg

Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

The bacteriophage λ regulatory protein, cII, has been purified and shown to activate positively RNA transcription from the two phage promoters which coordinately regulate phage lysogenic development. To obtain this protein, the cII gene was cloned into a plasmid vector carrying the strong, regulatable λ phage promoter P_L such that it was overproduced to levels approaching 5% of cellular protein.

WHEN bacteriophage λ infects *Escherichia coli* cells, the phage can either grow lytically, resulting in the formation of new progeny virus particles which are released on lysis of the host cell, or the phage may enter a state of lysogeny, in which the viral DNA integrates into the host genome and the expression of lytic functions is repressed. In normal physiological conditions, a balance between lytic and lysogenic growth is achieved and appreciable numbers of cells enter both developmental pathways¹⁻⁴.

The establishment of the lysogenic state requires the synthesis of two phage functions—repressor (cI), a protein which inhibits transcription of lytic functions, and integrase (int), a protein which catalyses the integrative recombination between the viral DNA and the host genome. The production of both proteins is positively regulated by the phage gene products cII and cIII and also affected by various host-encoded functions^{5,6}. The synthesis of repressor and integrase is coordinately controlled at the level of transcription from the two λ promoters P_E and P_I (Fig. 1). The DNA structure of these two sites has been determined⁷⁻¹⁰ and their location precisely defined^{11,12}. Little is known, however, about the precise roles of the cII and cIII gene products in this activation process. In particular, it is not known whether cII works alone or in combination with other factors or whether cII works directly or indirectly to activate transcription from P_E and P_I . Only by purifying the components and reconstituting the positive activation system *in vitro* can we hope to elucidate the role of cII and understand positive activation at these two promoters.

Cloning a 'lethal' gene function

During a normal λ infection, cII protein is synthesized for only a short time and seems to be rapidly turned-over¹³. Thus we set out to clone the λ cII gene into a plasmid vector in such a way that the gene would be efficiently and continually expressed. The general approach involved inserting a 1,300-base pair (bp) DNA fragment carrying the cII gene into a plasmid vector, in such a way that the cII coding region was positioned in proper orientation downstream from an efficient promoter signal for RNA transcription. We reasoned that efficient transcriptional expression of cII from the multicopy vector would result in high-level cII production. The purified 1,300-bp DNA fragment^{7,14} was inserted into several different derivatives of pBR322, each of which contained a known promoter signal of

either phage or bacterial origin positioned upstream from the site of insertion. Recombinants (Amp^r) were screened by size and restriction analysis for the presence of the fragment. Among multiple independent isolates (>10) which contained the insert, all had the fragment positioned with the cII coding region in opposite orientation to the direction of transcription. It appeared that transcriptional expression of the fragment gave rise to some lethal function.

This contention was supported by the facts that cleavage of the fragment at the single *HincII* restriction site within the cII gene now allowed each subfragment to be cloned downstream from the promoter signal, and that the entire fragment was readily inserted in both orientations into a pBR322 derivative lacking the promoter.

To circumvent these problems we reasoned that the cII gene (as well as any other lethal function) would have to be cloned such that its expression could be regulated. Thus we utilized a pBR322 derivative, pKC30, which contained a λ DNA fragment carrying the highly efficient promoter signal P_L (Fig. 1). This promoter can be regulated by the λ repressor protein (cI), a product which is synthesized continually and is regulated auto-genously in an *E. coli* λ lysogen¹⁵. A *HpaI* restriction site unique to pKC30 (Fig. 1) is located 321bp downstream from the start site of P_L transcription. The 1,300-bp DNA fragment carrying the cII gene was inserted into this site and transformants (Amp^r) were selected in an *E. coli* λ lysogen. In contrast to our initial results, recombinants were now obtained carrying the intact 1,300-bp DNA fragment positioned in both possible orientations relative to P_L -directed transcription. The desired recombinant, pKC30cII, carries the cII gene oriented correctly with respect to P_L transcription, whereas the recombinant pKC30cIIc carries the same fragment inserted in reverse orientation. When these plasmids were used to retransform both lysogenic and non-lysogenic *E. coli* strains, pKC30cIIc transformed both strains with high efficiency, whereas pKC30cII only transformed the lysogen with high efficiency and was unable to transform the non-lysogen. Apparently, the amount of repressor being synthesized in the single lysogen is sufficient to reduce cII expression in the pKC30cII derivative to a non-lethal level.

To express the cII gene product in the lysogen, it was necessary to induce (derepress) the P_L promoter, by using a lysogen which carried a temperature-sensitive mutation in the phage repressor gene (cI857)¹⁶. Thus, raising the temperature of these

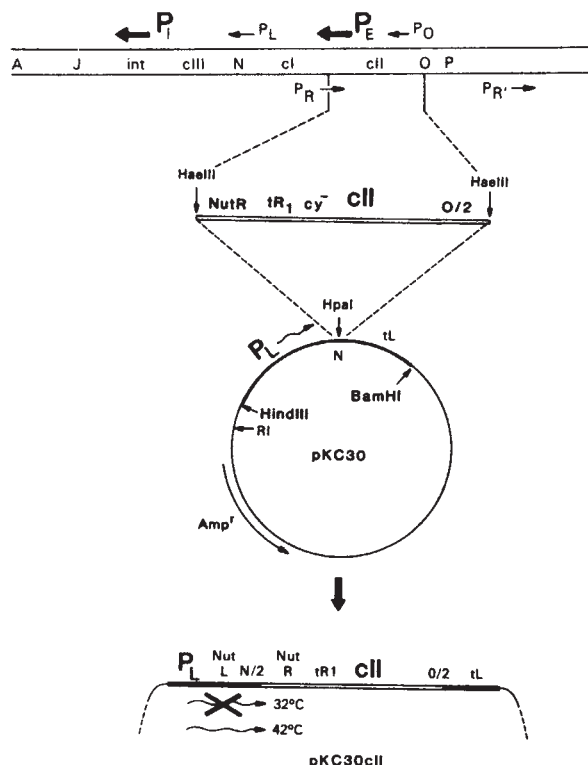


Fig. 1 Top: partial genetic map of bacteriophage λ . The locations of the positively regulated promoters, P_E and P_I , are indicated. These promoters are responsible for the coordinate expression of the phage lysogenic functions, repressor (cI) and integrase (int), respectively. Other major λ promoters are also shown (P_R , P_L , P_O , P_R'). Expanded below is the region encompassed by a 1,300-bp *HaeIII* DNA restriction fragment, which contains the *cII* gene and several other previously characterized phage regulatory sites (see below). Bottom: diagram of the construction of the plasmid vector which overproduces *cII* protein (pKC30cII). The *HaeIII* restriction fragment was inserted into the single *HpaI* restriction site which occurs on the plasmid pKC30. This fragment contains the proposed site of recognition for the antitermination function *N* (NutR), the rho-dependent transcription termination site (*tR1*), the P_E promoter mutation *cy3048* (*cy*⁻), the *cII* coding region and the amino-terminal half of the *O* gene (*O/2*)^{7,18,24,25}. The pKC30 plasmid is a derivative of plasmid pBR322 which contains a *HindIII*-*BamHI* restriction fragment derived from phage λ inserted between the *HindIII* and *BamHI* restriction sites within the tetracycline gene of pBR322 (R. N. Rao, unpublished data). The λ insert contains the promoter signal, P_L , another proposed site of *N* recognition (NutL), the *N* gene and the strong rho-dependent transcription termination signal, *tL*²⁰. The *HpaI* restriction site occurs within the *N* gene coding region²⁰. Purified *HaeIII* fragment (0.1 pmol) and *HpaI*-cleaved pKC30 plasmid (0.1 pmol) were blunt-end ligated²⁶ at 15°C for 14 h and released with *HpaI* after ligation. This DNA was used to transform a λ lysogen carrying a temperature-sensitive mutation (*cl857*) in its repressor gene. Amp^r recombinants were obtained and screened by size and restriction for the presence of the insert. Recombinants were obtained carrying the insert in both possible orientations (described in text). Note that the final pKC30cII construction contains the transcription regulatory sites, NutL, NutR and *tR1*, preceding the *cII* gene. Preliminary studies indicate that high-level protein expression requires that the lysogen carry a functional *N* gene which is induced by temperature (A. Shatzman and M.R., unpublished). This *N* gene product presumably functions at the Nut sites to antiterminate transcription at *tR1*.

cells (from 32 to 41 °C) inactivates the repressor, thereby turning on transcription from the P_L promoter (Fig. 1). We had now achieved the desired construction: the *cII* gene had been inserted into a high-copy number plasmid vector and was positioned downstream from a strong, regulated promoter signal.

High-level production and purification of the *cII* gene product

Lysogens carrying either the pKC30cII plasmid or the control plasmid pKC30IIc were examined for their ability to synthesize proteins in response to temperature induction. Cells were initially grown at 32 °C and then pulse-labelled with ³⁵S-Met before and at various times after induction at 42 °C (Fig. 2). Total cellular protein from each pulse was then characterized by electrophoresis on SDS-polyacrylamide slab gels. The results

(Fig. 2A) indicate that two major polypeptides (of molecular weights (M_r s) 10,500 and 32,000 respectively) are synthesized selectively in the pKC30cII lysogen in response to the temperature shift (pKC30cII lanes g-l). Neither of these polypeptides are made in the lysogen carrying pKC30IIc.

The molecular weight of the smaller peptide is appropriate to that expected for the *cII* gene product, as predicted by the DNA sequence of the *cII* coding region¹⁷. Moreover, this protein migrates identically with a polypeptide produced in a cell-free protein-synthesizing system (Fig. 2A, lane a) previously identified as *cII* (ref. 18 and C. Queen, unpublished results). The fact that the appearance of this polypeptide also depends on the correct orientation of the *cII* coding region and the temperature induction identifies this protein as the *cII* gene product.

The other polypeptide induced in the pKC30cII lysogen is probably a fusion peptide which derives from the *O* gene region of phage λ . The 1,300-bp DNA fragment inserted into the plasmid also contains information for the N-terminal 286 amino acids of the λ *O* gene¹⁹. The DNA sequence surrounding the *HpaI* fusion site²⁰ predicts that 14 additional in-frame translatable codons will be added to the 286 derived from the *O* gene before a translation termination codon occurs in frame. Thus, the P_L transcript from pKC30cII encodes a 300-amino acid *O* gene fusion product (32,000- M_r band) in addition to the *cII* gene product (the 10,500 band).

The intensities of the *cII* and *O* fusion products indicate that they are being synthesized at high rates relative to the other cellular products (Fig. 2A, lanes g, h). As *cII* is thought to be a relatively unstable protein¹³, we next examined whether the high rate of synthesis would lead to an accumulation of the *cII* protein in the cell. Again, lysogens carrying either pKC30cII or pKC30IIc were grown at 32 °C and temperature induced. After induction, total cellular protein was characterized on SDS-polyacrylamide gels and the major accumulated proteins detected by staining (Fig. 2B, lanes c, d). The *cII* and *O* fusion products were readily identified and found as major accumulated products in the pKC30cII lysogen (Fig. 2B, lane c). In these conditions *cII* protein was estimated to represent some 4-5% of total cellular protein. The *cII* protein was purified to ~98% homogeneity using standard procedures (Fig. 2B, lanes a, b). A detailed description of the isolation procedure and physical characterization of the protein will be given elsewhere (Y. Ho, H. S. and M. R., in preparation).

The procedure used here to clone, overproduce and obtain the *cII* protein (as well as the *O* gene fusion peptide) should have general application to the production and isolation of other proteins in bacterial cells. Of particular interest might be those products whose overproduction proves lethal to the cell²¹. The fact that the multiple copies of the highly efficient P_L promoter can be regulated in the single-copy lysogen allows cells to be grown initially without expression of the cloned function and then subsequently induced to produce the gene product. The rapidity of the induction procedure (1-1.5 h for maximum *cII* production) ensures maximum expression of the function within a relatively short time period. The system is readily adaptable to the production of almost any gene product (prokaryotic, eukaryotic or synthetic) provided the DNA insert contains all the necessary information for translating the product in the bacterial cell. A similar cloning vehicle has been used to overproduce the bacterial *trpA* gene product²².

cII-dependent transcription from promoters P_E and P_I

We next examined the ability of RNA polymerase, in the presence and absence of pure *cII* protein, to interact with and initiate transcription from the P_E and P_I promoters. We initially used a nitrocellulose filter binding assay to monitor the interaction, because RNA polymerase binds to DNA fragments containing promoters and forms heparin-resistant complexes which can be trapped on the filter²³. Our assay results (Table 1) indicated that RNA polymerase alone cannot form a stable initiation complex at the P_E site. In fact, the DNA fragment

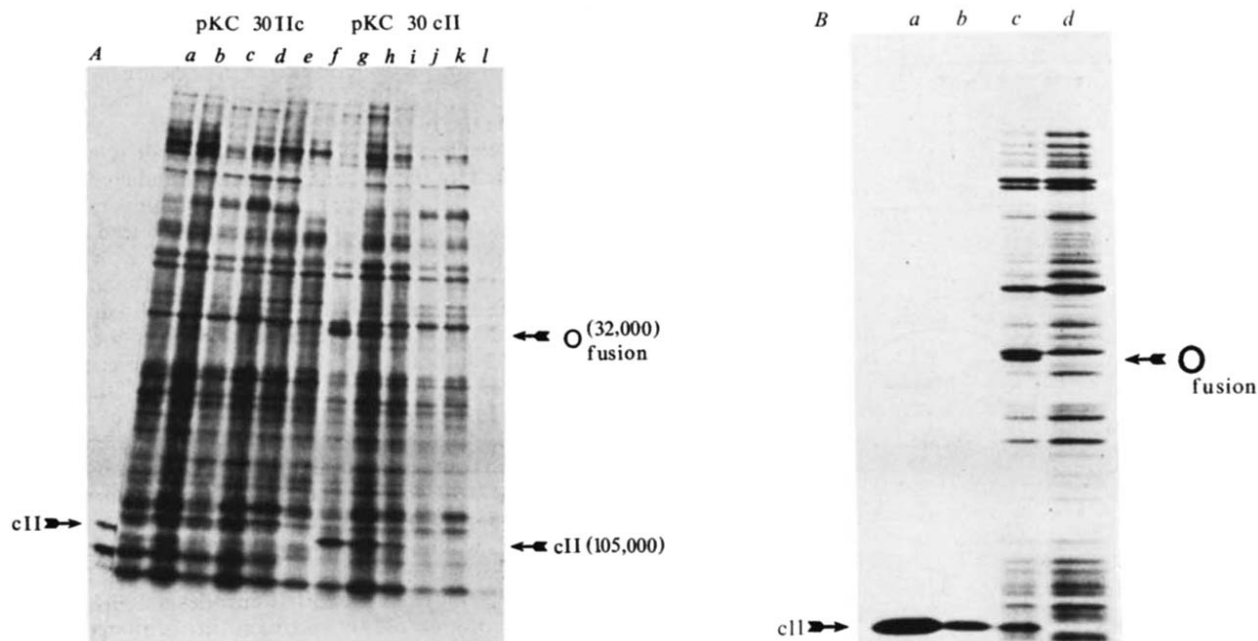


Fig. 2 A, autoradiogram of an SDS-polyacrylamide gel electrophoretic analysis of pulse-labelled proteins made in a λ c1857 lysogen carrying either pKC30cII (lanes g–l) or pKC30IIc (lanes a–f) at various times after heat induction. Cells were grown at 32 °C in minimal media (M56 supplemented with 0.5% glycerol and 25 $\mu\text{g ml}^{-1}$ ampicillin)³⁷. At $A_{650} = 0.35$ the temperature was raised to 42 °C. Aliquots (50 μl) of these cells were pulse-labelled for 45 s with ^{35}S -methionine (Amersham, 1,000 Ci mmol^{-1}) immediately before (lanes f, l) and at 5 (lanes e, k), 10 (lane d, j), 20 (lane c, i), 30 (lane b, h) and 40 (lanes a, g) min after heat induction. After pulse labelling, the cells were frozen (dry ice bath) and precipitated with trichloroacetic acid (final concentration 10%). The precipitate was resuspended in sample buffer (30 μl , 5.0 M urea, 0.1 M dithiothreitol, 1% SDS), boiled at 95 °C for 5 min, and subjected to electrophoresis on a polyacrylamide gradient slab gel (10% acrylamide, 0.25% bisacrylamide to 26% acrylamide, 0.325% bis-acrylamide) containing 0.1% SDS. The gels were dried and fluorographed³⁸. Lane a contains an ^{35}S -labelled marker polypeptide synthesized in a cell-free protein-synthesizing system and previously identified as the cII gene translation product (ref. 18 and C. Queen, unpublished data). Molecular weights are shown in parentheses. B, an SDS-polyacrylamide gel analysis of proteins accumulated in a c1857 lysogen carrying either pKC30IIc (d) or pKC30cII (c) following a 40-min heat induction. Cells were initially grown at 32 °C as described above and at $A_{650} = 0.7$, the temperature was raised to 42 °C for 40 min. Aliquots of these cells were analysed as above except that after electrophoresis the gel was stained with Coomassie brilliant blue R-250. The amount of cII was estimated by scanning the stained gel with a laser scanning densitometer (Zeineh). To purify the cII protein, cells transformed with pKC30cII plasmid were induced for 40 min at 42 °C. Cells were pelleted (8g), frozen and thawed, and then resuspended in 20 ml of buffer A (50 mM Tris-HCl, pH 7.6, 10 mM Na₂EDTA) supplemented with egg-white lysozyme (Worthington, 0.5 mg ml^{-1}). After 20 min at 4 °C, the cells were centrifuged at 30,000g for 20 min. The supernatant was precipitated with 40% ammonium sulphate and dialysed against buffer B (40 mM Tris-HCl, pH 7.6, 2 mM MgCl₂) containing 30 mM NaCl. The sample was loaded on a DEAE-Sephacel (Pharmacia) column (2.5 cm $\times$ 10 cm) equilibrated with buffer B containing 30 mM NaCl and eluted with a NaCl gradient in buffer B. Aliquots of each column fraction were analysed by SDS-gel electrophoresis and the fractions containing the 10,500- M_r band (see text) were pooled and precipitated with ammonium sulphate. The precipitate was resuspended in 0.5 ml of buffer B containing 0.1 M NaCl and fractionated by Sephacryl S-200 (Pharmacia) gel filtration column (1 cm $\times$ 35 cm) equilibrated with the same buffer as sample. Aliquots of each column fraction were again monitored by SDS-gel electrophoresis and the fractions containing the 10,500 band were pooled. Samples containing 1 μg (b) and 10 μg (a) of the final preparation of cII protein were analysed as described above.

Table 1 Effect of cII protein on initiation complex formation

DNA fragment	P_E (cy ⁺)	P_E (cy3048)	P_E (cy3008)	P_O
	(% c.p.m. retained on nitrocellulose filter)			
RNA polymerase	8	6	9	87
RNA polymerase + cII	75	8	7	–
cII protein	6	2	5	–

The 1,300-bp DNA fragment (Fig. 1) was cleaved with restriction endonuclease *HincII* and the products labelled at their 5' termini with T4 polynucleotide kinase (Boehringer-Mannheim) and [γ - ^{32}P]ATP (ICN, 2,000 Ci mmol^{-1})³⁴. The resulting fragments of 400 and 900 bp, which contain the P_E and P_O promoters respectively, were separated by electrophoresis on a 4% polyacrylamide slab gel and recovered in pure form by electroelution. End-labelled fragments were prepared identically from either λ wild-type DNA (cy⁺) or from mutant derivatives of λ carrying single base pair changes in the P_E promoter signal (cyL3048 and cyR3008, Fig. 5 and ref. 25). Transcription initiation complexes²³ were formed by incubating the appropriate fragment (in the presence of 0.5 μg carrier DNA) with RNA polymerase (1.0 μg , Enzo Biochemicals) and/or cII protein (0.3 μg) in a 50 μl transcription reaction mixture (25 mM Tris-HCl pH 7.9, 80 mM KCl, 10 mM MgCl₂, 1 mM dithiothreitol) lacking the four nucleoside triphosphates. Reactions were incubated for 10 min at 37 °C and then heparin (final concentration 100 $\mu\text{g ml}^{-1}$) was added and after 2 min the mixture passed through a nitrocellulose filter (Schleicher and Schuell B6). The filter was washed with 500 μl of transcription buffer and counted, and the percentage of counts retained on the filter determined (100% = 5,000 c.p.m.).

containing P_E was not filter bound by either RNA polymerase or cII protein alone, whereas a control fragment carrying the phage promoter, P_O , was retained. When RNA polymerase and cII protein were combined, however, the P_E fragment was retained on the filter, suggesting that cII protein is required for RNA polymerase interaction at P_E . To prove that the binding was P_E specific, the experiment was repeated using the same DNA fragment obtained from two different phage derivatives carrying P_E point mutations^{24,25}. One is a single base change in the –10 region of the promoter (cyL3048) and the other is a base change in the –35 region of the promoter (cyR3008). Each mutation eliminated filter binding (Table 1). Thus, the cII-dependent RNA polymerase interaction is specific for the wild-type P_E site.

We also examined the effect of cII on the ability of RNA polymerase to initiate transcription from either the P_E or P_I promoters in a purified *in vitro* transcription reaction. Transcriptions were carried out either in the presence or absence of purified cII protein using several different DNA templates. Two of these templates were constructed by inserting a λ DNA fragment carrying either P_E or P_I into the plasmid pBR322 (Fig. 3). Before transcription each template was cut with a restriction endonuclease such that RNA polymerase initiating at either P_E or P_I and proceeding to the end of the fragment would give rise to a discrete 'run-off' transcript. For each template a major RNA transcript of the expected size was obtained and its appearance depended completely on the presence of cII in the reaction (Fig. 4a, b). Several other discrete RNA products were obtained which apparently derive from other promoter signals contained on these plasmid derivatives (such as P_{amp} , P_{tet} , P_{rep} ,

Table 2 5'-end analysis of P_E and P_I RNAs

	P _E		P _I	
	α - ³² P label		α - ³² P label	
	GTP	ATP	UTP	CTP
T ₂	pppAp* (0.6) pppGp (0.4)	ND	pppUp* (1.8) pppUp (1.0)	pppUp* (0.2)
P ₁	pppG	ND	pppU (1.0)	—
T ₁	ND	(p)ppApGp	(p)ppUUCAG	ND

The P_E and P_I transcripts were labelled *in vitro* with the indicated [α -³²P]-nucleotides and gel-purified. After recovery from the gel, the following analyses were performed. RNA was digested in separate reactions with ribonucleases P₁ and T₂ and the products resolved by one-dimensional electrophoresis on DE81 paper at pH 1.7 (refs 27, 35). The products were identified by their relative mobilities using appropriate markers. The numbers given in parentheses indicate the relative molar yield of each product (determined by scintillation counting of the product after its detection by autoradiography). RNA was also digested with T₁ ribonuclease and the products separated by two-dimensional fingerprinting procedures²⁷. The first dimension was electrophoresis on strips of Cellogel (Kalex) at pH 3.5. The second dimension was homochromatography on TLC plates of DEAE cellulose (Analtech) using homosolvent B. The 5'-terminal oligonucleotide was identified after digestion of each T₁ oligonucleotide with pancreatic ribonuclease and electrophoretic separation of the products in one dimension on DE81 paper at pH 3.5 (ref. 27). ND, not determined.

* Indicates the labelled phosphate.

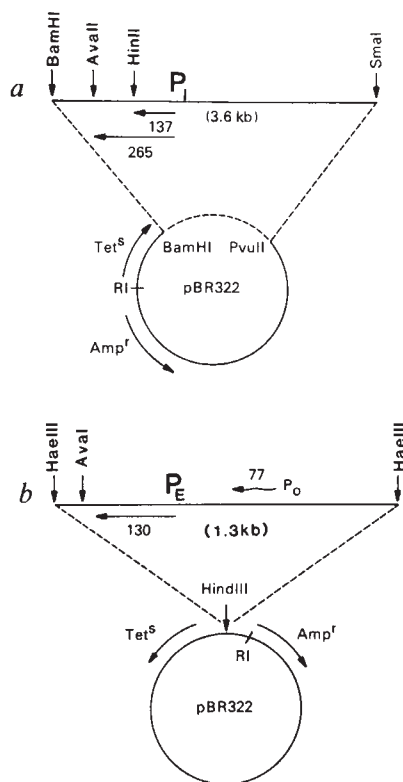


Fig. 3 Diagram of the plasmids used as DNA templates for *in vitro* RNA transcription. *a*, The 3,600-bp *SmaI*-*Bam*HI λ DNA fragment containing P_I was inserted between the *Pvu*II-*Bam*HI site of pBR322. This plasmid was constructed in our laboratory by U. Schmeissner (unpublished data). *b*, The 1,300-bp *Hae*III fragment (Fig. 1) containing P_E was inserted into the *Hind*III restriction site of pBR322 using *Hind*II synthetic linkers (Collaborative Research). Note that this fragment also contains the P_O promoter from which the 77 nucleotide-long RNA is transcribed (Fig. 4a, c and d)^{7,39}. Restriction enzyme cleavage sites are indicated and their respective distances from the P_I and P_E promoters noted when important to the description in the text.

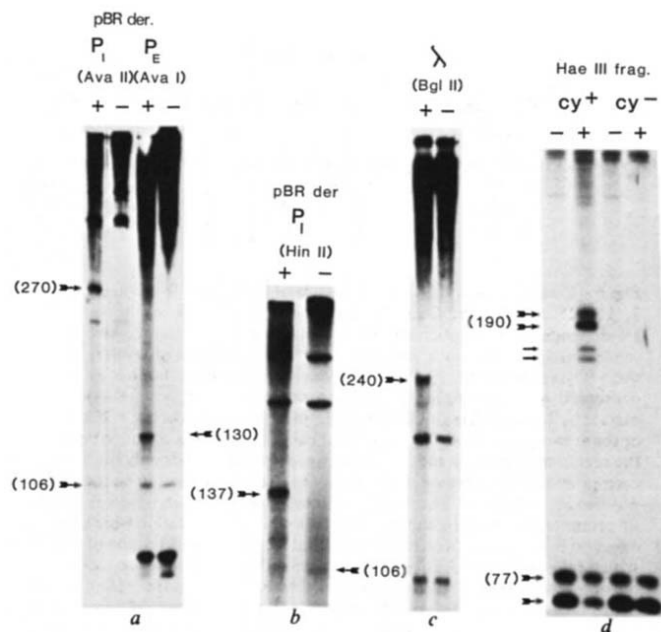


Fig. 4 Autoradiograms of polyacrylamide-gel electrophoretic analyses of ³²P-labelled RNA transcripts synthesized *in vitro* from several different DNA templates either in the presence (+) or absence (-) of purified cII protein. Molecular weights ($\times 10^3$) are shown in parentheses. *a*, The pBR322 derivatives shown in Fig. 3a (carrying P_I) and *b* (carrying P_E) were cleaved with restriction endonucleases *Ava*II and *Ava*I, respectively, and used as templates. The cII-dependent 270 nucleotide-long P_I transcript and the 130-nucleotide-long P_E transcript are indicated. Also indicated is the 106 nucleotide-long transcript which derives from the P_{rep} promoter located near the replication origin of the plasmid⁴⁰. *b*, The pBR322 derivative shown in Fig. 3a (carrying P_I) was cleaved with *Hin*II and used as template. The cII-dependent 137 nucleotide-long P_I transcript is indicated. The longer discrete transcripts seen in this gel are RNAs which presumably initiated at P_{amp} and P_{tet} promoters. *c*, λ DNA was cleaved with *Bgl*II and used as template. The cII-dependent 240 nucleotide-long P_E-initiated RNA is indicated. *d*, The purified 1,300-bp λ *Hae*II restriction fragment (Fig. 1) obtained from either wild-type phage (*cy*⁺) or phage carrying the cy3048 point mutation in the P_E promoter (*cy*⁻) was used as template. The cII-dependent 190 nucleotide-long P_E transcript is indicated as well as the 77 nucleotide-long RNA. All DNA templates were transcribed and analysed on 4 or 5% polyacrylamide slab gels which contained 8 M urea as previously described⁷. The transcription reaction mixture (50 μ l; as in Table 1 legend) contained the DNA template (0.5–2 μ g), *E. coli* RNA polymerase holoenzyme (3–5 μ g; Enzo Biochemicals) the four nucleoside triphosphates (each at 200 μ M) and [α -³²P]ATP, -UTP or -GTP (Amersham, specific activity 300 Ci mmol⁻¹). When present, cII protein was added to a final concentration of 10–20 μ g ml⁻¹ and all reactions were carried out at 37 °C for 20 min.

Fig. 4). These transcripts were unaffected by the presence of cII in the reaction. Thus, cII selectively activates transcription from the P_I and P_E promoters.

The dependence of P_E transcription on cII was also demonstrated using a phage λ DNA template. Total λ DNA was cleaved with restriction endonuclease *Bgl*II, which cleaves λ six times²⁶. One of these cleavage sites occurs 240bp downstream from P_E (ref. 14). *In vitro* transcription of the *Bgl*II-cut template resulted in the appearance of a cII-dependent RNA product ~240 nucleotides long (Fig. 4c). Two other well characterized λ RNA transcripts were also resolved from these reactions: the 77 nucleotide-long P_O-initiated RNA and the 192 nucleotide-long P_R-initiated RNA⁷. The transcription of both these RNAs was unaffected by cII. In an analogous experiment using λ DNA cut with restriction endonuclease *Ava*II (which cuts 265 bp downstream from P_I), we observed the predicted cII-dependent P_I-initiated transcript (data not shown).

We also examined the effect of a point mutation in the P_E promoter on the ability to synthesize the cII-dependent transcript. The 1,300-bp *Hae*III DNA restriction fragment (Fig. 1) was obtained either from wild-type λ (*cy*⁺) or from a mutant derivative (*cy*L3048), and used as template. Transcription of the *cy*⁺ DNA resulted in a major cII-dependent transcript ~190 nucleotides long (actually a doublet, Fig. 4d). The size of this RNA is consistent with the distance between P_E and the *Hae*III

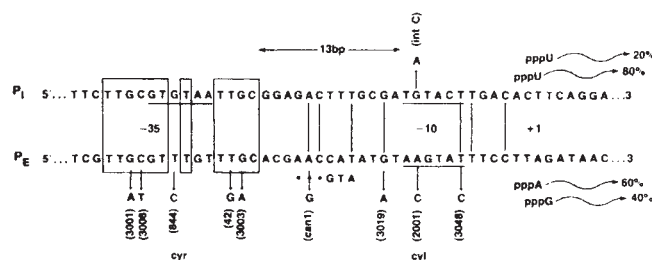


Fig. 5 Comparison of the DNA sequence of the P_1 (refs 8–10) and P_E (refs 7, 11, 25) promoters. Only the non-template strands are shown. The cII -dependent transcription startsites used *in vitro* are indicated. Also shown (underlined) are the sequences in the -10 region of both promoters and in the -35 region of the P_1 promoter which exhibit some homology to the conserved sequence found in other promoters for *E. coli* RNA polymerase³². The boxed regions indicate 11 of 14 base pairs in the -35 regions of these two promoters which are identical. Note that the distance between the region of homology and the transcription startsites is exactly the same in each promoter. This region of homology has been proposed to be the site of positive activation^{11,25}. The various *cyr* and *cyl* mutations which eliminate P_E promoter function are indicated and also the *can1* mutation which has no effect on P_E promoter function but does not alter the second codon of the cII structural gene²⁵. The ATG initiation codon cII translation is also shown (on the opposite strand). The *intC* mutation^{8,9} which occurs in the -10 region of the P_1 promoter and confers cII -independent, constitutive expression of *int* gene is designated.

end of the fragment. Two-dimensional T_1 -fingerprint analyses identified both of the RNAs in the doublet as transcripts which initiated at the P_E site. The *cy* point mutation eliminated all cII -dependent P_E transcription (Fig. 4d).

Note that the cII requirement for transcription from both the P_1 and P_E sites seems absolute. This is in contrast to transcription observed at other promoters which are positively regulated (such as P_{lac} , P_{gal} , P_{RM})^{27–29}. Even in the absence of the positive effector some level of transcription, albeit low, is usually observed. Moreover, this low-level transcription can be increased by including glycerol in the transcription reaction³⁰. This is not the case for P_E and P_1 ; addition of glycerol (up to 25% v/v) in the absence of cII protein resulted in no detectable P_1 or P_E transcription (data not shown).

Fingerprint identification and 5'-terminal nucleotide analyses

The identification of the *in vitro* synthesized cII -dependent RNAs as transcripts derived from the P_E and P_1 promoters was confirmed by two-dimensional T_1 -oligonucleotide fingerprint analysis (data not shown, see Table 2 and refs 7, 31). The T_1 oligonucleotides obtained from these fingerprints were compared and found to be identical to those predicted from the known DNA sequence of the regions immediately downstream from the P_E and P_1 promoters^{7–10}. In addition, we precisely positioned and quantified the 5'-terminal transcription startsites used *in vitro* by RNA polymerase at P_E and P_1 (Table 2). The results indicate that the cII -dependent P_E transcript initiates *in vitro* from the same sites used *in vivo*¹¹, predominantly with pppA (60%) and less often with the adjacent pppG (40%) (Fig. 5). Analogously, the cII -dependent P_1 transcript also initiates *in vitro* from two adjacent sites, one major (80%) and one minor (20%), using in both cases a UTP starting nucleotide (Fig. 5). Again, the *in vitro* startsite coincides precisely with that demonstrated *in vivo*¹² and constitutes the first demonstration both *in vitro* and *in vivo* of the use by RNA polymerase of a uridine triphosphate as the initiating nucleotide.

Conclusions

We have described the cloning, overproduction and isolation of the λ cII gene product and demonstrated the general potential of this vector system for overproducing gene products which may be either lethal to the cell or rapidly turned-over. We have also shown that cII protein alone functions as the positive effector and turns on transcription from the two phage λ promoters, P_E and P_1 , required for lysogenic development.

A variety of other phage and cellular factors have been

implicated in the transcriptional activation process of λ repressor and integrase expression^{2,3}. Speculations abound concerning the possible parts played by these various components in the P_E and P_1 activation mechanism. Our present finding, that cII protein and RNA polymerase alone are both necessary and sufficient to turn on P_E and P_1 transcription *in vitro*, strongly implies that these other factors are likely to exert their effects only in an indirect fashion.

The DNA structure which encodes the P_E promoter site also encodes the ribosome binding site information and the N-terminal coding region of the cII protein (Fig. 5). We previously speculated that if cII protein were the positive activator of P_E transcription, then its site of action would overlap with its own translational regulating and coding information⁷. This is now clearly the situation. We also proposed that the positive effector would probably interact at P_E in the -35 region^{11,25}, because of (1) the complete lack of any P_E -35 region homology with other known promoter sites³²; (2) the nature and positions of the -35 region mutations associated with P_E (*cyr* mutations); and (3) the extensive -35 region homologies exhibited between P_E and P_1 (Fig. 5). Recent experiments designed to probe the ability of cII to protect the P_E region from DNase cleavage (footprinting³³) indicate that cII is a DNA binding protein which interacts specifically in the -35 region of the P_E site (Y. Ho and M. R., in preparation). Apparently our ability to obtain cII in large quantity and demonstrate its function as a positive effector molecule now allows us to study directly the potential interactions of this protein with itself, the DNA template and RNA polymerase.

We thank Dr R. N. Rao for providing us with the plasmid pKC30, Dr U. Schmeissner for construction of the pBR322 derivative containing the P_1 promoter site, C. Brady for technical assistance, R. Steinberg for photographic work, and P. Donoghue and G. Taff for typing and editing the manuscript.

Received 15 December 1980; accepted 7 April 1981.

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LETTERS TO NATURE

Soft γ -ray emission from the region of MCG8-11-11

F. Perotti*, A. Della Ventura*, G. Villa*,
G. Di Cocco†, R. C. Butler‡§, J. N. Carter‡
& A. J. Dean‡

* Istituto di Fisica Cosmica, C.N.R. Via Bassini, 15/A Milan, Italy

† Istituto T.E.S.R.E., C.N.R. Via De' Castagnoli, 1 Bologna, Italy

‡ Department of Physics, University of Southampton, Southampton SO9 5NH, UK

The galaxy MCG8-11-11 inside the 0.093 square degrees error-box of the X-ray source 2A0551+466 (ref. 1), was recently found to be a Type 1 Seyfert galaxy². A 40-arc error-box from the A3 experiment on HEAO 1 has confined the X-ray emission to the nucleus or bar of this 14-*m*, galaxy³. The redshift is $z = 0.0205$ (ref. 2), corresponding to a distance of 123 Mpc ($H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$). The 2-10 keV luminosity ranges from $5 \times 10^{43} \text{ erg s}^{-1}$ to $1.2 \times 10^{44} \text{ erg s}^{-1}$ (refs 1, 3). Hard X-ray emission above 20 keV from the region of MCG8-11-11 has been recently reported⁴, and the A2 experiment on HEAO 1 has provided for the first time the emission spectrum of this object up to 30 keV (ref. 5). During a balloon flight on 30 September 1979 from Palestine, Texas of the Milan/Southampton (MISO) low-energy γ -ray telescope, an excess at the 3.9σ level in the flux above 90 keV was detected from a region of the sky containing MCG8-11-11. The emission spectrum, evaluated in the energy range 0.02-19 MeV, has similar spectral characteristics to those observed with the same telescope and associated with the Seyfert galaxy NGC4151 (ref. 6). Both spectra show evidence for a high-energy cutoff close to 3 MeV and this feature could be interpreted on the basis of the 'Penrose-Compton scattering' process⁷. A large amount of the observed low-energy γ -ray diffuse background⁸ could be produced by a few per cent of the X-ray emitting Seyfert galaxies having a γ -ray luminosity comparable with that observed from the region of NGC4151 or MCG8-11-11.

The MISO telescope⁹ consists of a 'Compton-coincidence' detection system inside a semi-active shield. The sensitive area is about 560 cm² and the aperture is 3° FWHM in both the azimuthal and zenithal planes. For the September 1979 flight, the energy range was set to be 0.05-19 MeV. A passively shielded hard X-ray detector (20-280 keV) having an effective area of 600 cm² and the same field of view of 3° FWHM was mounted parallel with the main telescope. A two-axis orientation system was used to point the telescope with a precision of about 0.3°.

The region of the sky containing MCG8-11-11 was studied on 30 September 1979 between 8 h 34 min UT and 14 h 59 min UT. Five drift-scans were performed to survey the region contained within the coordinate points RA 5 h 14 min and 6 h 33 min and centred on dec +46.2°. The mean float altitude during the on-source periods was equivalent to an atmospheric pressure of about 4.2 mbar and the telescope was set at zenith angles between 16° and 33°.

To minimize systematic variations in the background due to changes in the zenith and azimuth of the telescope, the data for each scan were analysed separately and the results statistically combined. The only significant source of systematic variation in the background was found to be linearly related to the changes of the residual atmospheric pressure between 3.7 and 4.5 mbar. For each separate scan the background, corrected for the atmospheric pressure variation, was subtracted from the

measured on-source counting-rate. A mean excess of 3.3 ± 0.85 c.p.s. above 90 keV (2.9 ± 0.8 c.p.s. above 260 keV from the γ -ray telescope) was found when the data from the five drift-scans were combined.

In Fig. 1a, the correlation between the counting-rate excess above 260 keV and the angular distance between the estimated direction of MCG8-11-11 and the axis of the MISO telescope is shown. The error-box for the observed γ -ray excess is plotted in right ascension and declination (for the 1950 equinox) in Fig. 1b together with the 4U and HEAO 1 (A2)⁵ error-boxes. The Ariel 5 error box¹ has about the same dimension of the filled circle showing the position of MCG8-11-11. The limits of our error-box are represented by the 1σ points along the scanning direction, and by the total aperture of the telescope (6°) in the orthogonal direction, which represents the 100% probability of containing the emitting object. The uncertainty due to the pointing system has been included. The only X-ray source within our error-box, even if extended up to the 4σ level, is MCG8-11-11, apart from the OSO 7 source 1M0600+46 (ref. 10) for which the projection on the sky of the 1.14 square degrees error-box is not available.

The same method of analysis was used to evaluate the contribution of the source in individual energy-loss channels of both the main telescope and the hard X-ray detector, between 20 keV

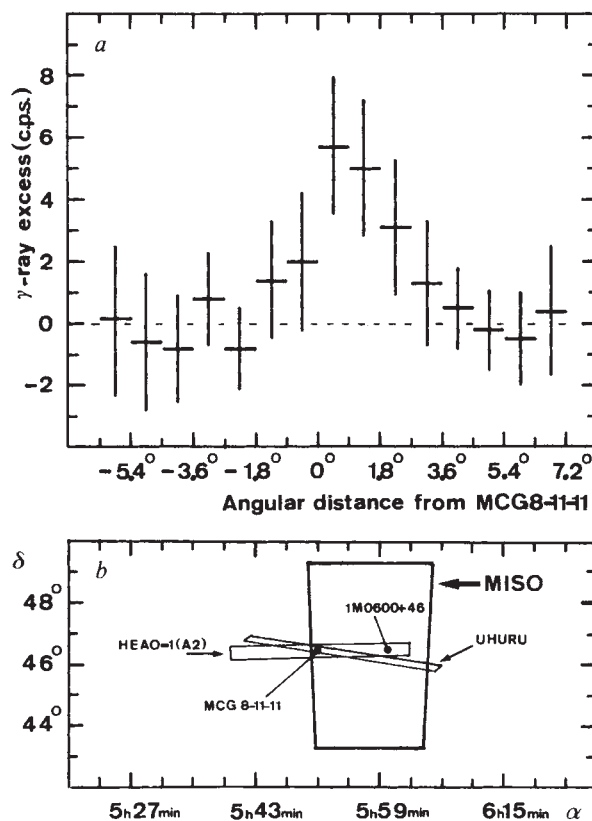


Fig. 1 a, γ -ray excess count rate above 260 keV versus the angle between the axis of the MISO telescope and the estimated direction of MCG8-11-11. b, The MISO error-box in right ascension and declination (for the 1950 equinox) is shown together with other astronomical data. The error-box of the OSO 7 X-ray source 1M0600+46 (ref. 10) is not available. The Ariel 5 error-box of the X-ray source 2A 0551+466 (ref. 1) has about the same dimension of the filled circle showing the position of MCG8-11-11. The MISO error-box has 68% probability across the scan direction and 100% across the other. The uncertainty due to the pointing system has been included.

§Present address: Istituto T.E.S.R.E., Via De' Castagnoli, 1 Bologna, Italy.

and 19 MeV. A photon spectrum at the top of the atmosphere was obtained by means of a matrix inversion technique which makes no *a priori* assumptions about the final shape of the emission spectrum. The absorption of photons in the residual atmosphere, the redistribution of photon energies through Compton interactions and pair-production, and the energy resolution of the detectors were taken into account. The error in this evaluation was estimated to be less than 10%. This photon spectrum is shown in Fig. 2 together with other measurements above 1 keV from the same region of the sky.

Our 2σ upper limits between 20 and 90 keV impose a severe constraint on the data fit at greater energies. In fact, an attempt to fit our data between 90 keV and 19 MeV with a single power-law photon spectrum leads to an estimated flux below 90 keV, a factor of two greater than the corresponding 2σ upper limits and to a reduced $\chi^2 = 2.77$ with 4 degrees of freedom ($\sim 2.5\%$ probability). Therefore, in a power-law representation of our spectral data a break, followed by a steeper spectrum, is needed between 2 and 4 MeV. At lower energies, the best fit is given by

$$I(E) = 7.9 \times 10^{-3} E^{-(1.0 \pm 0.7)} \text{ photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$$

corresponding to a reduced $\chi^2 = 1.45$ with 2 degrees of freedom. At higher energies the slope is about 3.8 if compatibility with the SAS 2 upper limit¹¹ is required.

In principle, we cannot exclude the possibility that the OSO 7 X-ray source 1M0600 + 46 (ref. 10) might be associated with the Seyfert galaxy MCG8-11-11. On the other hand, the OSO 7 error-box for this source is not available and therefore it is impossible to estimate the probability that the two X-ray sources are associated.

Assuming that we have detected a γ -ray emission from MCG8-11-11 and taking into account that its X-ray flux varies by a factor of 2 to 3 without evident spectral index variation^{2,12}, the HEAO 1 (A2)⁵ and MISO spectra could be in reasonable agreement.

The observed spectral feature at MeV energies is similar to that of the Seyfert galaxy NGC4151, in which a cutoff close to 3 MeV has been indicated by the MISO telescope observation⁶. The existence of the high-energy cutoff in the emission spectrum of these two Seyfert galaxies supports the suggestion¹¹ that a steepening of the spectrum between X-ray and γ -ray energies may be a general characteristic of the active extragalactic objects. This suggestion was based on the comparison between some measured X-ray spectra of active galaxies (for example, MKN501, Cen A, 3C273) and the corresponding positive results or intensity upper limits above 35 MeV.

A model has been recently proposed⁷ in which the cutoff at MeV energies is expected to be a 'universal' feature of the emission by active nuclei of galaxies, provided that a rapidly spinning Kerr black hole ($M > 10^8 M_\odot$) surrounded by a hot ($T \sim 10^9$ K) accretion disk exists within these nuclei. In these conditions, the Penrose-Compton scattering mechanism¹³ stochastically generates γ -ray bursts with energies up to 3 MeV. The spectral shape is expected to be flatter than the X-ray spectrum in the tens of keV region and the burst duration is about $\Delta T \sim (2.2 \text{ h}) \times 10^{-8} M/M_\odot$. Since the luminosity of MCG8-11-11 in the energy range 0.09-3 MeV, as obtained from our data, is $L(123 \text{ Mpc}) = 7 \times 10^{46} \text{ erg s}^{-1}$, on the basis of this model a lower limit of $5 \times 10^9 M_\odot$ is expected for the mass of the black hole. Thus, the γ -ray burst duration should be greater than 4.6 days, consistent with no evident flux variation during the 6.5 h observation of MCG8-11-11 by the MISO telescope. If we have detected a γ -ray emission from both the Seyfert galaxies NGC4151 and MCG8-11-11 during a balloon flight, the bursts must be rather frequent, at least during some periods of higher activity in these galaxies.

The spectrum of the cosmic γ -ray background⁸ shows a feature at MeV energies similar to that observed from the regions of MCG8-11-11 (Fig. 2) and NGC4151 (ref. 6). Moreover, the γ -ray background has a photon spectral index of about 1.7, not inconsistent within the statistical errors with those

observed at γ -ray energies from these two galaxies. Assuming that the present data pertain to MCG8-11-11, the contribution of Seyfert galaxies to the diffuse γ -ray background in the energy range 0.09-3 MeV can be estimated. The X-ray luminosity function for Seyfert galaxies obtained by the HEAO 1 (A2) experiment leads to a local volume emissivity above $L_{X\min}$ of¹⁴

$$B_X(2-10 \text{ keV}) = 2 \times 10^{38} \left(\frac{L_{X\min}}{10^{43}} \right)^{-0.75} \text{ erg s}^{-1} \text{ Mpc}^{-3}$$

Taking a γ -ray background emissivity up to 3 MeV of $B_\gamma = 1.8 \times 10^{39} \text{ erg s}^{-1} \text{ Mpc}^{-3}$ (ref. 15), the ratio of the two emissivities is

$$B_\gamma/B_X = 9(L_{X\min}/10^{43})^{0.75}$$

Since $L_{X\min}$ is in the range of $(2-5) \times 10^{42} \text{ erg s}^{-1}$ (refs 14, 16), then

$$B_\gamma/B_X = 2.7-5.4$$

For MCG8-11-11 the ratio between the 0.09-3 MeV γ -ray luminosity and the 2-10 keV X-ray luminosity is:

$$\begin{aligned} L_\gamma/L_X(\text{MCG8-11-11}) &= \frac{7 \times 10^{46} \text{ erg s}^{-1}}{(0.5-1.2) \times 10^{44} \text{ erg s}^{-1}} \\ &= (6-14) \times 10^2 \end{aligned}$$

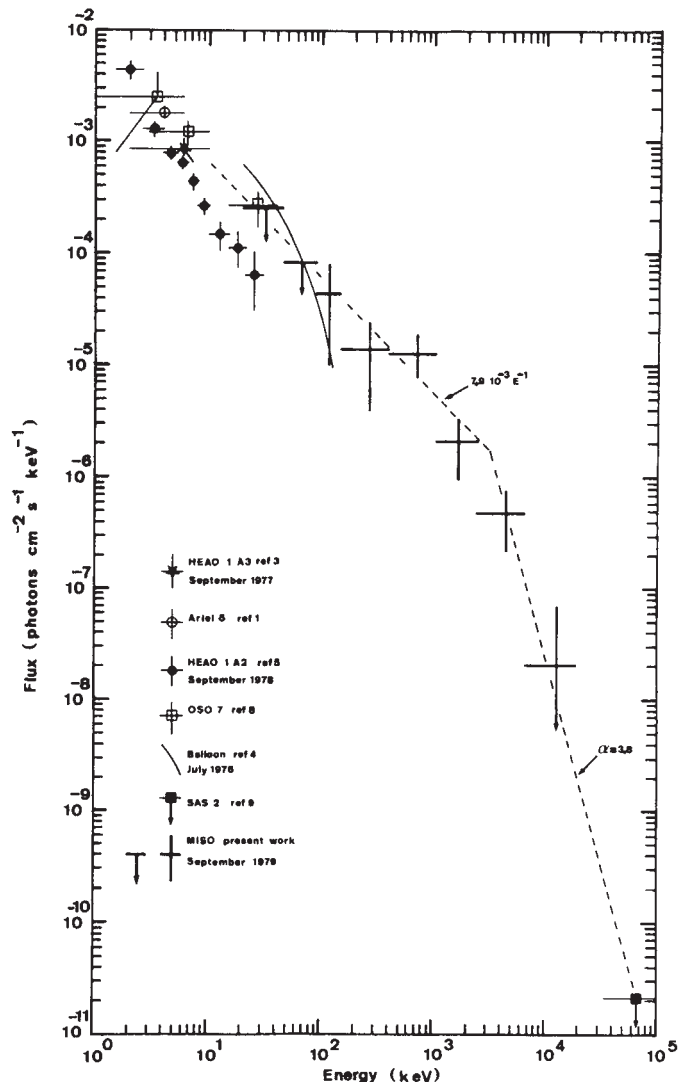


Fig. 2 The photon spectrum observed by the MISO telescope from the region of MCG8-11-11 is shown together with other measurements above 1 keV from the same region of the sky. When available, the date of observation is given. The dashed line represents the best fit power-law of the MISO data with a cutoff at about 3 MeV. The solid curve (ref. 4) represents the fit obtained by the authors assuming a bremsstrahlung spectrum.

For NGC4151 the observed γ -ray luminosity is $(0.5-2) \times 10^{45} \text{ erg s}^{-1}$ (refs 6, 17) and the 2–10 keV X-ray luminosity is in the range $(3.5-7) \times 10^{42} \text{ erg s}^{-1}$ (refs 16, 18), giving:

$$L_{\gamma}/L_X(\text{NGC4151}) = (1-5) \times 10^2$$

Consequently, we could assume $L_{\gamma}/L_X = 10^2$ as a characteristic ratio for this sample. The comparison between $L_{\gamma}/L_X = 10^2$ and $B_{\gamma}/B_X = 2.7-5.4$ implies that few per cent of all the Seyfert galaxies with L_X (2–10 keV) $> L_{X\text{min}} = (2-5) \times 10^{42} \text{ erg s}^{-1}$ could produce the observed diffuse background in the 0.09–3 MeV energy band. And since the γ -ray background produced by Seyfert galaxies cannot exceed the total cosmic background, only a few per cent of Seyferts can have γ -ray luminosities as high as those observed from NGC4151 and MCG8–11–11.

We thank E. Boldt and D. Leiter for helpful comments and suggestions on this paper.

Received 19 February; accepted 1 May 1981.

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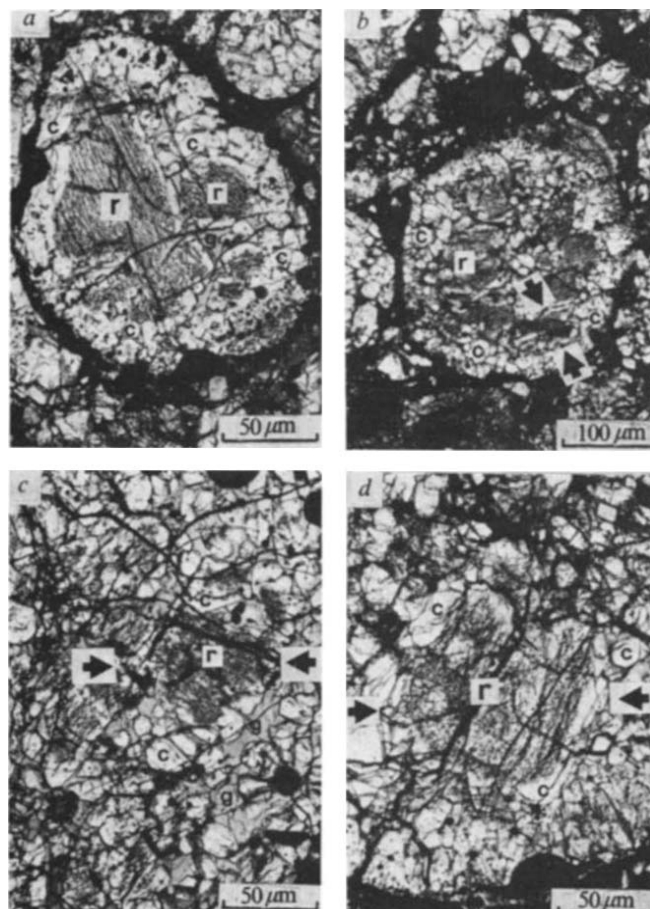


Fig. 1 a, Chondrule 2-1 containing a relic olivine. r, Relic olivine showing large, dirty and anhedral appearance; c, crystallized olivine showing small, clear and euhedral appearance; g, glass. b, Chondrule 2-2. Scanning profile of MgO across the grain between the arrows is shown in Fig. 2b. c, Closed view of relic olivine in chondrule 2-3. Clear part around the relic olivine is overgrown olivine. Scanning profile between the arrows is shown in Fig. 2c. d, Closed view of chondrule 2-4. Scanning profile between the arrows is shown in Fig. 2a.

Evidence for secondary origin of chondrules

Hiroko Nagahara

Geological Institute, University of Tokyo, Hongo, Tokyo 113, Japan

Although chondrules have been extensively studied chemically^{1,2}, petrologically³⁻⁵ and experimentally⁶⁻⁸, their origin is still not certain. I discuss here whether chondrules were condensed directly from the primitive solar nebula⁹⁻¹¹ or were formed from precursory materials by various mechanisms, such as high velocity impact of small bodies^{12,13}, impact on the surface of a parental body^{14,15} or dust fusion¹⁶⁻¹⁸. Investigations of an Antarctic meteorite suggest that chondrules could have formed through melting of pre-existing materials.

The Antarctic meteorite ALH-77015(L3) has closely packed chondrules which show wide variations in texture, bulk chemical composition and mineralogy (work in preparation). They are classified into four textural groups; barred, radial, glassy and porphyritic chondrules. The porphyritic chondrules are most common and show wide variations in their crystal/liquid (groundmass) ratios and crystal shapes. Several chondrules contain two types of olivine. One, which has numerous dusty inclusions and a fuzzy 'dirty' appearance, is always found near the centre of the chondrules as a large anhedral grain, or as disaggregated small anhedral grains (Fig. 1a, b). The other is euhedral small grain which exists around the former olivine and has clear feature. This clear olivine also overgrows the 'dirty' large olivine crystal (Fig. 1c, d).

These two types of olivine have different chemical compositions. Figure 2a shows the zonal pattern of MgO across the dirty and clear olivines in chondrule 2-4 (Fig. 1d). The dirty olivine shows 'reversal' zoning where the core is more enriched in iron than the rim. In contrast, the clear olivine shows 'normal' zoning

where the core has more magnesium than the rim. It is impossible to form both types of olivine from a liquid during a single crystallization process. The dirty and relatively iron-rich olivine is probably a relic mineral which was originally rich in iron and was not completely melted when it was heated. On the other hand, the clear olivine seems to have crystallized directly from the liquid, which was probably formed by melting of the pre-existing olivine, pyroxene and plagioclase. The fact that the overgrown rim of the relic olivine has the same composition as the clear one (Fig. 2a) supports this hypothesis. Figure 2b, c show the zonal patterns of MgO in the relic olivine with overgrown rim in chondrules 2-2 (Fig. 1b) and 2-3 (Fig. 1c). The overgrown olivine has more magnesium than the relic part, and there is compositional discontinuity between them.

The two types of olivine are also distinguished by their minor element content. Figure 3 shows the CaO content compared with $X_{\text{Mg}}(\text{Mg}/\text{Mg} + \text{Fe})$ of the two olivines in four chondrules. In all cases, crystallized olivine contains more CaO than the relic olivine. In general, the olivine that crystallized at high temperature or that crystallized rapidly contains more CaO (ref. 19). This fact supports the suggestion that the clear olivine is crystallized from the liquid when the chondrules were heated and that the dirty olivine is a relic mineral which had been equilibrated at lower temperatures before chondrule formation.

Relic olivine is the first direct evidence for the presence of precursory material in the formation of chondrules; that is at

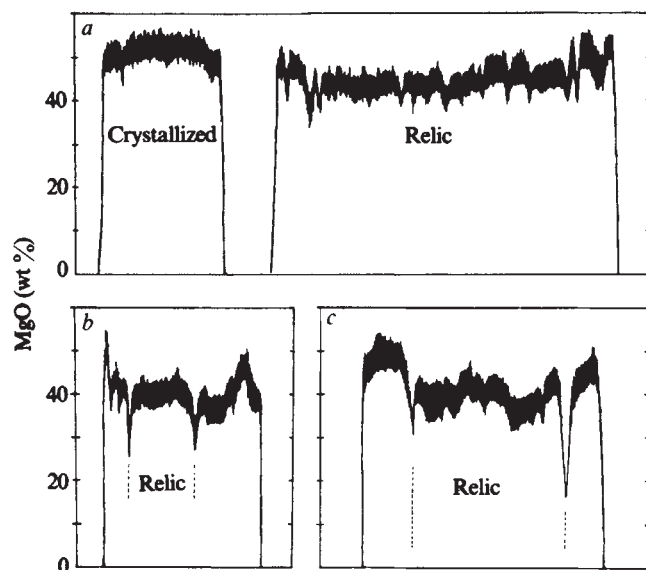


Fig. 2 *a*, Scanning profile of MgO in crystallized olivine and olivine in chondrule 2-4. Scanning position of relic olivine is shown in Fig. 1*d*. Chemical composition of rim of the two olivines is equal. *b*, Chondrule 2-2. Scanning position is shown in Fig. 1*b*. Overgrown part contains more MgO than the relic part. *c*, Chondrule 2-3. Scanning position is shown in Fig. 1*c*.

least some chondrules were not condensed directly from the gas of primitive solar nebula. In the present case, the relic olivine is so large that it can easily be distinguished from the crystallized olivine. However, if the relic olivine were small and/or were nearly completely melted, it would be difficult to distinguish relic minerals from the crystallized ones.

Some chondrules were, therefore, not necessarily crystallized from the completely melted liquid. In this case, the maximum temperature of chondrule formation would be lower than the liquidus temperature of chondrules, which is about 1,300–1,700 °C (ref. 8), and be consistent with recent experimental results (refs 8, 20, and H. N. and A. Tsuchiyama, in preparation). Dodd⁴ has discussed whether the microporphyritic chondrules were formed by fragmentation of rocks which crystallized from liquids and whether they are different in origin from the remelted chondrules such as barred or radial ones. Although his model seems to explain the difference in chondrule

texture, it does not account for microporphyritic texture which is difficult to form from wholly melted materials. The existence of pre-existing materials in the nucleus is an effective explanation for both the formation of porphyritic or microporphyritic minerals (H. N. and A. Tsuchiyama, in preparation). The fact that the bulk composition of the chondrules, which were clearly crystallized from the liquid, are mixture of olivine, pyroxene and plagioclase²¹ also strongly suggests that such chondrules at least were formed by melting of pre-existing materials.

I thank Professor I. Kushiro for discussions and reading the manuscript, also T. Nagata and K. Yanai for providing the sample.

Received 17 March; accepted 1 May 1981.

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Prebiotic atmospheric oxygen levels

J. H. Carver

Research School of Physical Sciences, Australian National University, Canberra ACT 2600, Australia

It has been argued^{1–2} that the early Precambrian atmosphere contained a negligible amount of oxygen and that the transition to the modern oxygen-rich atmosphere began some 2,000 Myr BP when photosynthetic oxygen produced by blue-green algae oxidized ferrous iron in solution and escaped from the ocean to the atmosphere. Others³ have argued for an even earlier occurrence of an oxygen-rich atmosphere. Although photosynthesis has provided the oxygen of the present atmosphere, the photosynthetic model for Precambrian oxygen production has difficulties. There is the problem of identifying the large deposits of reduced carbon needed to account for the red beds⁴ and the predominant role of photosynthesis as an oxygen source in the early Precambrian has been questioned on both biochemical and geochemical grounds⁵. Before photosynthesis the only possible abiotic source of atmospheric oxygen would have been the direct photodissociation of water vapour by solar UV photons. What atmospheric oxygen level could have been produced by this purely abiotic process? It has been argued⁶ that the abiotic oxygen level was entirely negligible, $<10^{-12}$ PAL (present atmospheric level), but this assumed that the water vapour content of the palaeoatmosphere was the same as at present with a constant H₂O mixing ratio of 3.8 p.p.m. above an altitude of 10 km. I argue here that this is too restrictive an assumption and calculate possible atmospheric oxygen levels for larger water vapour mixing ratios which cannot be ruled out by our limited knowledge of Precambrian conditions. Even a moderate oxygen content for the early Precambrian atmosphere could have had significant evolutionary implications because a biologically effective UV ozone screen would be established once the oxygen content exceeded 10^{-2} PAL (refs 7–11).

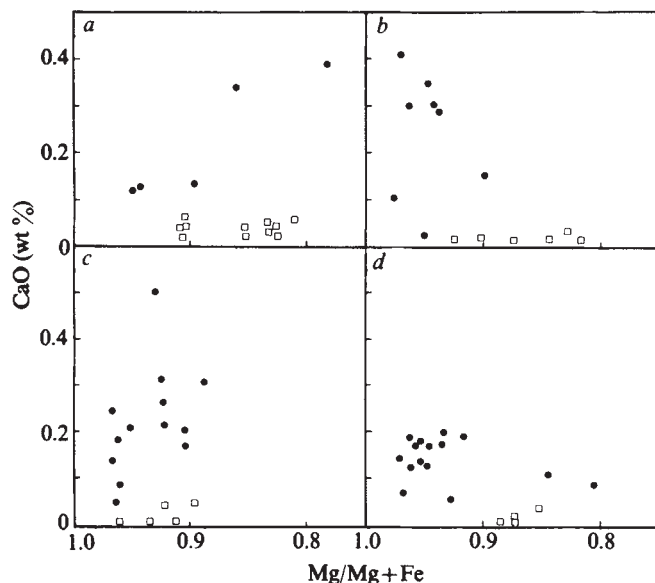


Fig. 3 CaO content in relic (□) and crystallized olivine (●) in four chondrules. *a*, Chondrule 2-1; *b*, chondrule 2-2; *c*, chondrule 2-4; *d*, chondrule 2-3.

In abiotic conditions the production of free oxygen in excess of atmospheric and surface oxidation would have depended on the amount of water vapour in the lower palaeoatmosphere and on transport processes that governed the escape rate of hydrogen through the exosphere. Abiotic production of atmospheric oxygen is limited by transport rather than by the water vapour photodissociation rate and, provided the exospheric temperature exceeds ~ 500 K, the hydrogen escape rate is given approximately by the limiting flux condition¹²⁻¹⁴

$$\phi = \frac{b}{H} f_{\text{tot}} \quad (1)$$

where b is the binary diffusion parameter, H is the atmospheric scale height and f_{tot} is the total hydrogen mixing ratio at the homopause. Kasting *et al.*¹⁵ have noted that the limiting flux condition may not apply exactly for palaeoatmospheres having high hydrogen escape rates ($f_{\text{tot}} \geq 10^{-3}$) and low mesospheric oxygen contents ($\leq 10^{-5}$ PAL) owing to the partial ineffectiveness of ion-molecule reactions in converting H_2 into H in the thermosphere. Throughout most of the range of present interest, however, the limiting flux condition should provide a sufficiently good approximation.

For the Earth's atmosphere, $b/H \approx 2 \times 10^{13} \text{ cm}^{-2} \text{ s}^{-1}$ and the present stratospheric water vapour content of ~ 4 p.p.m. imply a hydrogen escape rate of $\sim 1.6 \times 10^8 \text{ H atoms cm}^{-2} \text{ s}^{-1}$. This is less than the current hydrogen emission rate from volcanoes estimated⁶ as $V \approx 2.5 \times 10^8 \text{ H atoms cm}^{-2} \text{ s}^{-1}$. Thus abiotic processes are now a negligible source of atmospheric oxygen and a net production of oxygen by photodissociation could have occurred in the past only for palaeoatmospheres containing significantly more water vapour than does the present atmosphere. It is unlikely that a highly reducing atmosphere could have survived for long under the photolytic influence of the solar UV radiation and it will be assumed that after an initial period of rapid outgassing the palaeoatmosphere¹⁶ consisted mainly of carbon dioxide, water vapour and nitrogen with a volcanic emission rate comparable to the present value. With these assumptions the net hydrogen escape rate is

$$\psi = \phi - V \approx (2 \times 10^{13} f_{\text{tot}} - 2.5 \times 10^8) \text{ H atoms cm}^{-2} \text{ s}^{-1} \quad (2)$$

Larger values of f_{tot} imply increased rates of hydrogen escape (and hence oxygen production) but the oxygen level achieved depends on the loss rate. For the assumed palaeoatmosphere that does not contain large quantities of reduced gases the major loss process would be surface oxidation once the hydrogen escape rate exceeded the volcanic emission rate. Oxidation rates in present conditions probably vary as some fractional power of the atmospheric oxygen content¹⁷ because they depend on the rate of exposure of surface materials as well as on the intrinsic properties of those materials. At low oxygen levels in a palaeoatmosphere the supply of oxygen rather than the exposure of fresh material would have been the limiting factor and, following Brinkmann¹⁸, the oxidation rate L is assumed to have been proportional to the oxygen content $n(\text{O}_2)$

$$L = \frac{n(\text{O}_2)}{\tau} \quad (3)$$

where τ is a characteristic time for surface oxidation. At equilibrium the loss rate L is equal to the oxygen production rate P that corresponds to the net hydrogen escape rate given by equation (2).

The equilibrium atmospheric oxygen levels calculated with $P = L$ are shown in Fig. 1 for $\tau = 0.1$ –100 Myr and $f_{\text{tot}} = 10^{-6}$ – 10^{-2} . Brinkmann¹⁸ investigated Precambrian oxygen production and surface oxidation for a range of τ between 1 and 100 Myr. Hart¹⁹ assumed that τ was ~ 20 Myr. The solid lines in Fig. 1 at τ values of 1 and 10 Myr bracket the present oxidation time estimated by Holland as 3 Myr.

The very dry stratosphere of the present atmosphere^{20,21} is considered to result from circulation and condensation through the cold tropical troposphere with a typical temperature of ~ 195 K corresponding to a saturated humidity of ~ 4.5 p.p.m. Because of the approximately exponential dependence of humidity on temperature the water vapour content of the

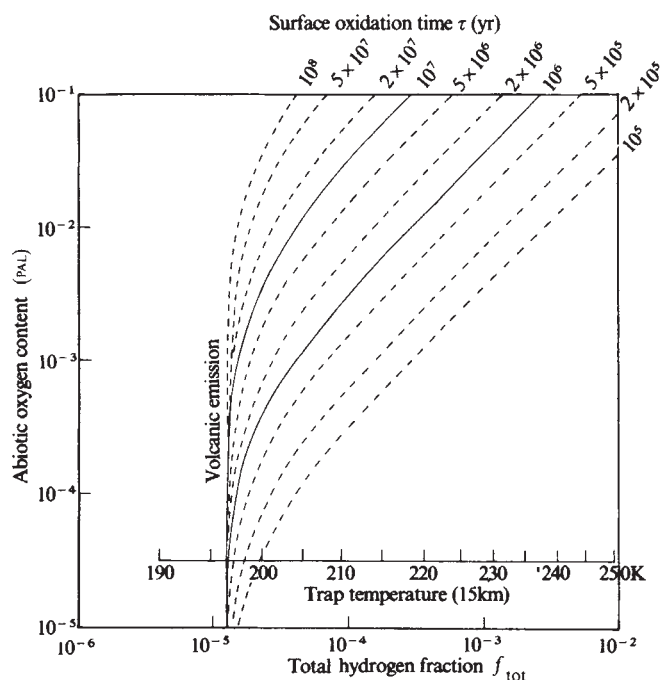


Fig. 1 Equilibrium abiotic oxygen content (PAL) as a function of surface oxidation time (τ yr) and total hydrogen content (f_{tot}) in the lower atmosphere. Temperatures that would produce a water vapour content equal to f_{tot} for a notional cold trap at 15 km are indicated. The solid lines bracket estimates of the present surface oxidation time constant. Note that the atmospheric oxygen content is negligible unless f_{tot} exceeds its present value so that exospheric hydrogen escape overwhelms volcanic hydrogen input.

stratosphere would vary rapidly with changes in the temperature of the atmospheric cold trap. If, for example, the polar tropopause at ~ 225 K were the controlling temperature the stratospheric water vapour content would increase by nearly two orders of magnitude. Figure 1 also shows notional 'trap temperatures' corresponding to particular values of f_{tot} even though the palaeoatmosphere may not have had a true tropopause.

It is unlikely that the palaeoatmosphere would have possessed the particular combination of temperature profile and circulation pattern that produces the very dry stratosphere of the present atmosphere. That substantial changes in the radiation balance of the atmosphere have occurred during geological time is implied by the fact that the Earth's surface temperature has remained within the range 0 – 100 °C (as indicated by geological evidence for the continued presence of large amounts of liquid water) even though theories of stellar evolution imply that the Sun has brightened²² by 30 – 40% since it first joined the main sequence some $4,500$ Myr ago. Sagan and Mullen²³ suggested that an ammonia greenhouse might have kept the Earth's mean surface temperature above the freezing point of seawater but it is unlikely that an ammonia-rich atmosphere could have persisted throughout the early Precambrian and a more probable explanation is that the greenhouse effect was provided by a CO_2 rich palaeoatmosphere^{24,25}. Other possible explanations for the non-freezing of the oceans under the weak early Sun are changes in the albedo or the rotation rate of the Earth²⁶. Any such explanation would profoundly affect the temperature profile and water vapour content of the palaeoatmosphere.

The range of total hydrogen contents, f_{tot} , shown in Fig. 1 corresponds to notional cold trap temperatures at 15 km between 190 and 250 K. Figure 1 only indicates a range of possibilities for the equilibrium abiotic oxygen content of a palaeoatmosphere and any conclusions based on our meagre understanding of the Precambrian environment must be very tentative. The available evidence does, however, suggest that the Precambrian lower atmosphere may have been warmer and moister than at present so that significant quantities of atmos-

pheric oxygen could have been produced by abiotic means. Surface temperatures as high as 52 °C (325 K) at 1,300 Myr BP and 70 °C (343 K) at 3,000 Myr BP are suggested by the chert measurements of Knauth and Epstein²⁷. Schopf²⁸ has summarized evidence indicating Precambrian ocean surface temperatures of 50–70 °C. Model atmosphere calculations^{24,25} also suggest that early Precambrian surface temperatures may have been warmer than at present owing to the atmospheric greenhouse effect provided by a dense carbon dioxide blanket. For oxygen deficient atmospheres the photochemical region extends well down into the lower atmosphere with the water vapour photolysis rate being a maximum at an altitude of ~5 km (ref. 6). The hydrogen escape rate in the limiting flux condition is set by the water vapour content at the 10–15 km level and above ~20 km hydrogen molecules replace water vapour as the dominant hydrogen species. Even if the atmosphere is very cold above 20 km it will not affect the water vapour level that fixes the limiting flux.

Model atmosphere calculations suggest significant warming of the lower atmosphere in Precambrian conditions. The Precambrian models of Morss and Kuhn²⁹ indicate temperatures ~15 °C warmer than at present at 15 km which would imply $f_{\text{tot}} \sim 10^{-4}$ and $n(\text{O}_2) \sim 10^{-2}$ for present day surface oxidation rates and $n(\text{O}_2) \sim 10^{-3}$ for 10 times the present oxidation rate. These models, however, neglect variations in solar luminosity and more detailed calculations of palaeotemperature profiles are required before any very firm conclusions can be drawn from the models. Larger values of $n(\text{O}_2)$ are implied by the chert measurements of Knauth and Epstein²⁷ which indicate a possible surface warming of (40–50 °C). If this warming had extended throughout the lower palaeoatmosphere (if the lapse rate had been similar to the present one) the chert temperatures would imply total hydrogen fractions f_{tot} at 15 km in the range 10^{-3} – 10^{-2} . In these conditions equilibrium abiotic oxygen levels $n(\text{O}_2)$ could have reached 0.1 PAL unless surface oxidation rates were substantially greater than at present. Thus if the temperatures were as high as suggested by the model atmosphere calculations and by the chert measurements abiotic atmosphere oxygen levels could have approached 10^{-3} PAL (even for a surface oxidation rate 10 times the present) and may have reached 10^{-2} –0.1 PAL during any particularly warm and moist Precambrian conditions. Atmospheric oxygen in such amounts would have been sufficient to have produced a biologically effective Precambrian ozone shield^{7–11}. The present calculations are consistent with the geological results of Grandstaff³⁰ who has shown that the occurrence of detrital uraninite does not require an essentially anoxic early Precambrian atmosphere.

Received 10 March; accepted 4 May 1981.

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Surface viscosity of water

J. C. Earnshaw

Department of Pure and Applied Physics,
The Queen's University of Belfast, Belfast BT7 1NN, UK

The recent suggestion¹ that surface viscosity effects can arise from the molecularly diffuse nature of a fluid surface or interface has prompted this re-examination of data concerning capillary waves on the surface of water. Previous work² has shown that the propagation of such waves is not compatible with the predictions of accepted theory for a surface having no abnormal rheological properties. These apparent discrepancies can be resolved in terms of one of the surface shear viscosities predicted by Goodrich. The existence of such surface viscosities for pure water suggests that caution must be exercised in interpreting surface viscosities observed for surface films in terms of molecular structure of, or interactions within the films.

Capillary waves on a fluid interface propagate in a manner governed by the viscoelastic properties of the system. The evolution of the waves in time can be described (in two dimensions; x in the surface) by a vertical displacement

$$\zeta = \zeta_0 \cos(qx) \exp(\alpha t) \quad (1)$$

where q is the surface wave-vector ($2\pi/\lambda$) and α is a complex frequency:

$$\alpha = i\omega - \Gamma \quad (2)$$

In the case of clean liquid–air surface there is a well-established dispersion equation³ (based on linearized hydrodynamic theory) relating α to q and to the physical properties of the fluid. In first-order approximation this predicts a frequency

$$\omega^2 = \sigma_0 q^3 / \rho \quad (3)$$

and the damping constant

$$\Gamma = 2\eta_0 q^2 p \quad (4)$$

where σ_0 and η_0 are respectively the surface tension and the dynamic viscosity of the liquid. Non-propagating surface modes exist for sufficiently large q ; they are not of present concern. Higher-order approximations for ω and Γ can be derived; they essentially agree with exact numerical solutions of the dispersion equation^{2,4}. Careful experiments² on thermally excited capillary waves of small amplitude on a water–air interface do not agree precisely with this theory. The observations imply a viscosity η_0 rather above the accepted value for water. Modification of the dispersion equation to allow for the viscosity of air⁵ only partially removes these systematic discrepancies. It has not been clear to date whether the discrepancies arise from the linearization of the hydrodynamic equations or from some modification of the properties of water close to the surface. Persuasive arguments can be presented against the former possibility.

Recently Goodrich¹ has shown that the molecularly diffuse interfacial region between two fluids displays anisotropic momentum transport parallel to and normal to the interface; this anisotropy leads to surface viscosity effects. Goodrich identifies four surface viscosity effects, two (κ and κ_N) describing dilational viscosity within and normal to the interface and two (η and η_N) describing shear viscosity. These surface viscosity coefficients are usually associated¹ with the viscoelastic behaviour of a fluid interface supporting a surfactant monolayer.

There seems, however, to be no *a priori* reason why such effects should not arise for clean fluid surfaces or interfaces. Goodrich's theory thus justifies an examination of the second possible explanation mentioned above.

Surface viscosity effects due to surfactant monolayers exert well-understood effects on capillary waves³. The predominant effect of the additional viscous dissipation is to increase the effective damping of the waves. Goodrich^{1,6} shows that κ_N has no part in the linearized theory of capillary waves; further the shear modes decouple from the other modes and are not observed in the experiments mentioned above (these involve the scattering of light, which does not couple to surface shear modes). The hydrodynamic theory is generalized by introducing frequency dependence to the surface tension:

$$\sigma(\omega) = \sigma_0 + i\omega\eta_N \quad (5)$$

and a frequency dependent surface compressibility:

$$\varepsilon(\omega) = \varepsilon_0 + i\omega\kappa \quad (6)$$

Classically ε_0 , κ and η_N are all zero for clean surfaces but may assume non-zero values in the presence of a surface film. The spectrum of light scattered by surface waves reflects their temporal evolution (experiments directly yield values for ω and Γ); the theoretical form including surface viscosity effects has been calculated⁷ and can be used to estimate the viscoelastic properties of interfaces.

The effects of κ and η_N on ω and Γ are shown in Fig. 1; the two coefficients are seen to have very different effects. The increase in Γ caused by κ seems to saturate, whereas that due to η_N increases until eventually the capillary waves become over-damped. These effects of η_N are similar to those of an increased value of bulk viscosity, η_0 . The various surface viscosities have quite different effects on the dispersion properties of surface waves. Figure 2 shows that the relative effect of κ on Γ is approximately independent of q whilst that due to η_N increases with q . The effects of κ and η_N are comparatively small and rather large values were chosen for Fig. 2 to accentuate the general trends. The differences in behaviour of ω with q , if plotted on Fig. 2, would not be distinguishable (except for the higher η_N value at $q > 1,500 \text{ cm}^{-1}$).

Also shown in Fig. 2 are data² on Γ for waves on clean water-air surfaces; the data deviate increasingly from the classical assumptions ($\varepsilon_0 = 0$, $\kappa = \eta_N = 0$) as q increases. The wave frequencies are uniformly close to those for the classical

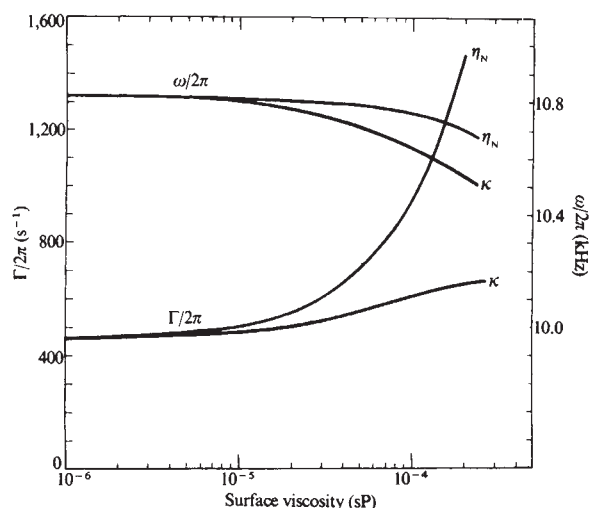


Fig. 1 The effects of surface viscosity (η_N and κ) on the frequency and damping constant of capillary waves ($q = 400 \text{ cm}^{-1}$) on water. Note the different scales for ω and for Γ .

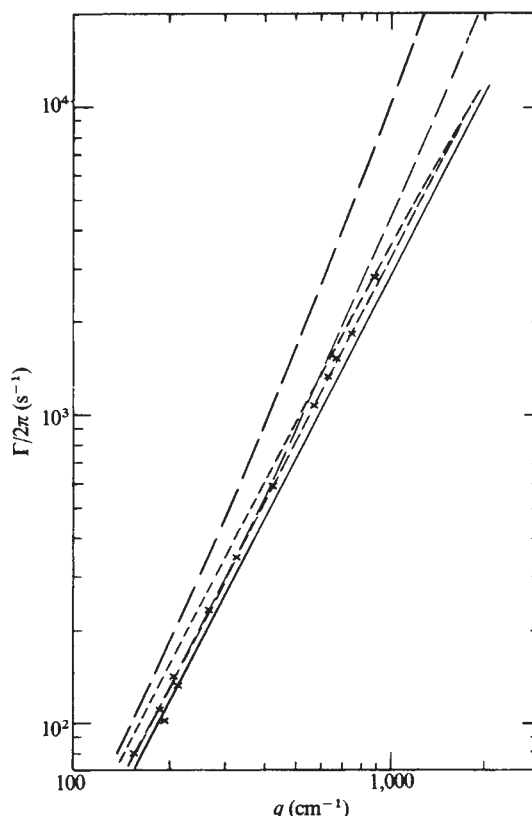


Fig. 2 The variation of Γ with q for water under various assumptions about the surface: —, classical surface, $\kappa = \eta_N = 0$; ---, $\eta_N = 0$ and $\kappa = 2 \times 10^{-5} \text{ sP}$ (lower line) and 10^{-4} sP ; — · —, $\kappa = 0$ and $\eta_N = 2 \times 10^{-5} \text{ sP}$ (lower line) and 10^{-4} sP . Also shown are the data of ref. 2 for water at 20°C .

assumptions². Taking as minimal assumptions the accepted values $\sigma_0 = 72.75 \text{ dyne cm}^{-1}$ and $\eta_0 = 1.002 \text{ cP}$ (appropriate to $T = 20^\circ \text{C}$) and $\varepsilon_0 = 0 \text{ dyne cm}^{-1}$, values for κ and η_N can be estimated using these data. The overall maximum likelihood estimates are $\kappa = 0 \text{ sP}$ (surface Poise— $\text{mN m}^{-1} \text{ s}$) and $\eta_N = 1.2 (\pm 0.4) \times 10^{-5} \text{ sP}$. The upper limit on κ is $1.5 \times 10^{-5} \text{ sP}$. The limits quoted in both cases are 95% confidence limits.

Unfortunately quantitative data for comparison with that of ref. 2 is scarce. Several studies of capillary waves on water have used similar experimental techniques to those of ref. 2. The tabulated data of Langevin⁸ are all compatible with the present conclusions apart from the lowest q value investigated, for which the instrumental correction to Γ is largest. Bird and Hills⁹ derive a bulk viscosity slightly above the accepted value. The data of Hård *et al.*¹⁰ yield estimates of η_0 below the accepted value; in this study, however, instrumental corrections were only made approximately and may have been overestimated. The damping of mechanically generated capillary waves on water⁴ seems to be consistent with the present conclusions.

Received 24 March; accepted 4 May 1981.

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Raman investigation of ring configurations in vitreous silica

Shiv K. Sharma

Hawaii Institute of Geophysics, University of Hawaii, Honolulu, Hawaii 96822, USA

Joseph F. Mammone* & Malcolm F. Nicol

Department of Chemistry, University of California, Los Angeles, California 90024, USA

Random network models of glass structures¹ provide satisfactory qualitative descriptions of many properties of glasses (see reviews in refs 2–4). However, to obtain good quantitative agreement between theoretical analyses and experimental observations^{5–11} it is often necessary to assume that specific ring structures in the random networks have special importance. In the case of vitreous silica ($v\text{-SiO}_2$), distributions of loops of SiO_4 tetrahedra in the random network have been invoked^{5–9} to match calculated and experimental X-ray radial distribution functions (RDF). High resolution X-ray photoelectron spectra¹² of $v\text{-SiO}_2$ and quartz also provide evidence for the occurrence of four-, six- and higher-membered rings of SiO_4 tetrahedra in $v\text{-SiO}_2$. Raman spectra are also sensitive to local microstructures in vitreous solids. We have therefore examined the question of rings in the structure of $v\text{-SiO}_2$ by comparing its Raman spectrum with spectra of crystalline silica polymorphs whose shortest loops contain four (coesite) and six (for example, α -quartz) tetrahedra. This comparison indicates that the sharp shoulder at 490 cm^{-1} in the spectrum of $v\text{-SiO}_2$, previously attributed to a defect structure³ or a longitudinal optic mode¹⁴, can be assigned to four-membered ring structure. We discuss here possible basis for the stability of four-membered rings of SiO_4 tetrahedra in $v\text{-SiO}_2$ at ambient pressure. Possible reasons for the absence of the 490 cm^{-1} band in the vibrational density-of-states derived for a random network model by Bell and co-workers^{12,15} are discussed.

Figure 1 shows first-order room-temperature Raman spectra of polycrystalline samples of α -quartz and coesite and a bulk sample of high purity $v\text{-SiO}_2$. These spectra were measured in the 90° scattering configuration with a Jobin-Yvon Raman spectrometer and 488-nm line of an argon ion laser¹⁶. The positions and spectral characteristics of the bands in these spectra are given in Table 1. Examination of these spectra suggests that the very strong band at 437 cm^{-1} and the sharp shoulder at 490 cm^{-1} in the spectrum of $v\text{-SiO}_2$ are related to the strong bands at 465 and 521 cm^{-1} in the spectra of α -quartz and coesite, respectively (Table 1).

Theoretical analyses^{17–20} of the vibrational spectrum of $v\text{-SiO}_2$ have indicated that the 437 cm^{-1} band belongs to $\nu_s(\text{Si-O-Si})$, the symmetric stretch. In this mode, the bridging oxygen moves along the line bisecting the Si-O-Si bond angle. The frequency of this mode is sensitive to both the Si-O-Si bond angle and the Si-O bond length^{18,19}. A third parameter which could affect $\nu_s(\text{Si-O-Si})$ is the short-range connectivity of the network structure. Raman data for framework aluminosilicates with four-, five- and six-membered rings of TO_4 tetrahedra ($T = \text{Si}$ or Al) indicate that, among crystals of isochemical compositions, the frequency of $\nu_s(\text{T-O-T})$ increases with reduction in the ring size (Table 2). This correlation implies that the band at 437 and the shoulder at 490 cm^{-1} in $v\text{-SiO}_2$ arise from tetrahedral network structures, composed of six- and four-membered rings, respectively. That these bands occur at lower frequencies in the spectrum of $v\text{-SiO}_2$ than in the spectra of the crystalline polymorphs is probably due to differences in Si-O-Si bond angles in the rings in $v\text{-SiO}_2$ and the crystalline

Table 1 Frequencies of the bands observed from Raman spectra

α -Quartz (cm^{-1})	Coesite (cm^{-1})	Silica glass† (cm^{-1})
—	77 s	68 s, bd, wp
128 s*	116 s	—
—	151 m	—
—	176 s	—
207 s, bd	204 m	—
—	220 w	—
264 m	269 s	—
—	314 m	—
—	326 m	—
356 m	355 m	—
395 m(sh)	—	—
—	427 m	—
465 vs	466 m	437 vs, bd, p
—	521 vs	490 s(sh), p
—	—	606 w, p
696 w	—	—
795 w(sh)	785 w	—
807 w	815 w	797 w, bd, dp
—	837 w	830 w(sh)
—	1,036 w	—
1,066 w(sh)	1,065 w	1,060 w, bd, dp
1,083 w	1,144 w	—
1,161 w	1,164 w	1,190 w, bd, dp
1,231 w	—	—

Measurement accuracy in the spectra of crystalline polymorphs, $\pm 2\text{ cm}^{-1}$ for weak band and $\pm 1\text{ cm}^{-1}$ for strong bands; in the spectrum of SiO_2 glass, $\pm 4\text{ cm}^{-1}$ for sharp and strong bands and $\pm 10\text{ cm}^{-1}$ for broad and weak bands.

* w, Weak; s, strong; vs, very strong; bd, broad; sh, shoulder; p, polarized; dp, depolarized.

† The arrow indicates the presence of a strong continuum between the band maxima.

polymorphs. Raman measurements of α -quartz under high pressures have shown that the frequency of the 464 cm^{-1} band is sensitive to compression²⁶. Studies of neutron irradiated $v\text{-SiO}_2$ also show that the frequencies of these bands increase on densification of the vitreous silica²⁷.

The large halfwidth of the 437 cm^{-1} band of $v\text{-SiO}_2$ (Fig. 1) is usually attributed to the dispersion of Si-O-Si angles. However, the position of $\nu_s(\text{Si-O-Si})$ also depends strongly on ring size (see Table 2). Thus, the $\nu_s(\text{Si-O-Si})$ vibrational modes of five-, seven- and higher-membered rings of SiO_4 tetrahedra, which may also be present in the random network, may be responsible for the broadening of the 437 cm^{-1} band.

The $v\text{-SiO}_2$ band at 606 cm^{-1} , which has been attributed to defects^{13,27}, has no counterpart in the spectra of silica polymorphs (Fig. 1). Our data, therefore, support its assignment to defect structures involving partially broken Si-O bonds in the network²⁸.

The 400–600 cm^{-1} region of the Raman spectrum of $v\text{-SiO}_2$ thus suggests that at least three different types of structural configurations occur in $v\text{-SiO}_2$. Each of these configurations, of course, will be associated with a range of Si-O-Si bond angles such that the material lacks long range order. The broad and complex bands in the 700–1,200 cm^{-1} region of the $v\text{-SiO}_2$ spectrum (Fig. 1) also may be attributed to the presence of at least three different types of structural groups in metastable equilibrium in $v\text{-SiO}_2$.

The presence of three different types of structural groups in $v\text{-SiO}_2$ can be further supported by the density and Raman data²⁷ on neutron irradiated $v\text{-SiO}_2$. Both the density and intensity of the 490 cm^{-1} band of $v\text{-SiO}_2$ increase on exposure to neutrons, and both properties show maxima at 5×10^{19} neutrons cm^{-2} . Neutron irradiation increases the intensity of the 606 cm^{-1} band at an even faster rate than the intensity of the 490 cm^{-1} band, but the 606 cm^{-1} intensity does not show a maximum. In contrast, the intensity of the 437 cm^{-1} band of unirradiated SiO_2 decreases with increasing exposure to neutrons²⁷. The different responses of the 437, 490 and 606 cm^{-1}

* Present address: Textile Fibers Department, Experimental Station, E. I. Du Pont de Nemours and Co., Wilmington, Delaware 19898, USA.

bands of v-SiO₂ to neutron irradiation clearly demonstrate that these bands belong to different species. The observed maxima in the intensity of the 490 cm⁻¹ band and in the density at 5×10^{19} neutrons cm⁻² can be attributed to the conversion of the five-, six- and higher-membered rings of SiO₄ tetrahedra into four-membered rings.

Recently, Mikkelsen and Galeener²⁹ reported that both the density and the intensity of the 606 cm⁻¹ band of v-SiO₂ (Suprasil W1) increase with the fictive temperature. The intensity of the 490 cm⁻¹ band was also enhanced as the fictive temperature of v-SiO₂ increased (see Fig. 1 in ref. 29), although the magnitude of the increase could not be precisely determined.

The increase of the intensity of the 606 cm⁻¹ band with increasing fictive density suggests either that a higher density structure is responsible for the 606 cm⁻¹ mode, or that, while most of the network relaxes to a denser than average structure, this is accompanied by an increase in the concentration of a less dense structure responsible for the 606 cm⁻¹ band²⁹. The Raman data²⁷ on neutron-irradiated v-SiO₂ clearly show that the 606 cm⁻¹ band belongs to a less dense structure. At exposures of 2×10^{22} neutrons cm⁻², the intensity of the 606 cm⁻¹ band is 1.4 times that for v-SiO₂ exposed to only 5×10^{19} neutrons cm⁻²; but the additional irradiation decreases the density to 2.518 g cm⁻³ from 2.560 g cm⁻³.

The intensities of the 490 and 606 cm⁻¹ bands increase with increasing fictive density as well as with increasing density of v-SiO₂ at low neutron irradiation ($\leq 5 \times 10^{19}$ neutrons cm⁻²); this suggests that there is a relationship between the four-membered ring structure responsible for the 490 cm⁻¹ band and the defect centres responsible for the 606 cm⁻¹ mode. The silicon atoms at these defects carry a formal positive charge. We suggest that the four-membered rings in v-SiO₂ at ambient pressure are stabilized by the excess positive charge on silicon atoms at the defect sites just as the four-membered rings of TO₄ tetrahedra in crystalline feldspar are stabilized by cations³⁰. This interpretation suggests that the intensities of both the 490 and 606 cm⁻¹ bands will initially increase with the density of v-SiO₂ at ambient pressure. However, the extent to which defect centres can stabilize four-membered rings at ambient pressure is limited as indicated by the maxima in both the intensity of the 490 cm⁻¹ band and the density of unirradiated v-SiO₂ at an exposure of 5×10^{19} neutrons cm⁻².

Bell *et al.*¹⁵ and Bell and Dean^{17,31} have computed vibrational densities-of-states and other properties of SiO₂ for large (~200 SiO₄ molecular unit) continuous random-network clusters of almost perfectly tetrahedral units. Within each cluster, molecular units interact through short range (nearest-neighbour) bond forces while the cluster interacts with non-bridging oxygens at its surface. Raman spectra of v-SiO₂ have recently been computed¹¹ in terms of the behaviour of much smaller sections (typically 10–20 molecular units) of Bell and Dean's clusters. Computations with these models have given fairly good

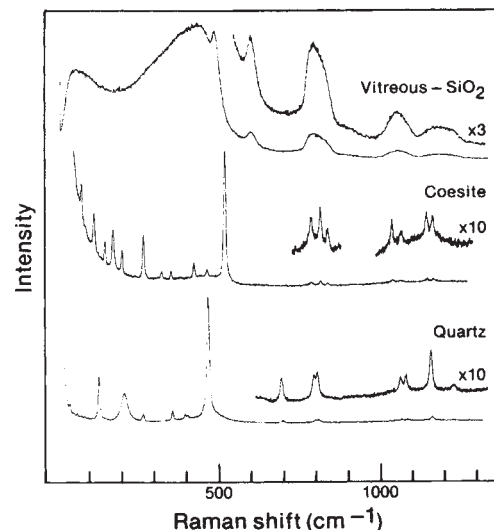


Fig. 1 Raman spectra of crystalline α -quartz, coesite and vitreous silica (v-SiO₂) at room temperature (laser 488.0 nm; slit and laser power at the sample used for crystalline polymorphs were 3 cm⁻¹ and 300 mW and for SiO₂ glass these were 5 cm⁻¹ and 500 mW, respectively).

agreement with observed X-ray and neutron diffraction data. Calculated spectra also resemble, to a first approximation, observed Raman spectra of v-SiO₂. However, the sharp bands at 490 and 606 cm⁻¹ and the broad band at 1,200 cm⁻¹ do not have counterparts in the computed spectra^{14,32}. This discrepancy may result from two factors that the v-SiO₂ models of Bell and co-workers do not take into account: (1) the presence of defect centres involving partially broken Si–O bonds²⁸ in the interior of v-SiO₂ and (2) the ring statistics⁹, especially the presence of four-membered ring structures which seem to be stabilized by the defect centres. It is now possible to calculate vibrational spectra of vitreous solids on the basis of random network models containing defect centres^{10,33} and ring structures^{10,11}. The present results suggest that such calculations for v-SiO₂ should be attempted to extend our understanding of the Raman spectrum as well as the structure of vitreous silica.

Received 19 January; accepted 27 April 1981.

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Table 2 Relationship between $\nu_s(\text{T-O-T})$ frequency and ring structures in some tectosilicates

Minerals	$\nu_s(\text{T-O-T})$ in crystalline polymorphs	Repeat units and shortest rings of tetrahedra ²¹	Ref.
α -quartz	464 vs	6	22
β -quartz (700 °C)	464 vs	6	22
α -cristobolite	416 vs	6	23
Coesite	521 vs	4	Present work
LiAlSi ₂ O ₆ -III	480 vs	6	24
LiAlSi ₂ O ₆ -II	492 vs	5	24
Low albite (NaAlSi ₃ O ₈)	506 vs	4	25
Orthoclase (KAlSi ₃ O ₈)	513 vs	4	25
Anorthite (CaAl ₂ Si ₂ O ₈)	503 vs	4	25

Heat flow on the Hawaiian Swell and lithospheric reheating

R. S. Detrick*, R. P. von Herzen†, S. T. Crough‡, D. Epp§ & U. Fehn||

* Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02281, USA

† Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

‡ Department of Geosciences, Purdue University, West Lafayette, Indiana 47907, USA

§ Hawaii Institute of Geophysics, University of Hawaii, Honolulu, Hawaii 96822, USA

|| University of Rochester, Rochester, New York 14627, USA

Most oceanic volcanic centres caused by hotspot activity are surrounded by sea floor that is unusually shallow for its age^{1,2}. There is increasing evidence that swells found on older sea floor, like the Hawaiian Swell, have formed from a broad-scale reheating and thinning of the lithosphere as it passes over a mantle hotspot^{3,4}. If the formation and subsequent disappearance of these swells are controlled by thermal processes, they should have an above-normal heat flux. We present here the results from 95 new heat flow measurements on the Hawaiian Swell. Along the older part of the swell the heat flow is 20–25% higher than the normal heat flow for crust of this age. This anomaly is consistent with the observed swell uplift. The shape and amplitude of the heat flow anomaly require that the reheating be largely confined to the lower half of the lithosphere.

The Hawaiian Swell is a broad, elongate region of anomalously shallow depths surrounding the youngest portion of the Hawaiian–Emperor seamount chain (Fig. 1). The swell has a maximum width of ~1,200 km and extends more than 2,700 km WNW along the length of the Hawaiian chain. Southeast of Oahu the swell rises ~1.5 km above the surrounding sea floor. The height of the swell gradually decreases to the northwest along the older portion of the island chain. While various models have been proposed for the origin of the Hawaiian Swell^{5–7} it has also been argued^{3,4} that the swell forms as a result of a broad-scale reheating of the lithosphere by the Hawaiian hotspot. The main evidence for this model is that the disappearance of the swell seems to follow a predictable thermal

subsidence curve³. If the lithosphere has been reheated, the swell should have an above-normal heat flux with the size and location of the heat flow anomaly depending on the manner in which the lithosphere is reheated⁴.

To test this hypothesis we recently obtained 95 reliable heat flow measurements at eight well-sedimented sites along the Hawaiian Swell (Fig. 1)⁸. The measurements were made with the Woods Hole Oceanographic Institution's digital heat flow instrument using thermistors externally mounted on either a piston core or a multipenetration pogo probe. Thermal conductivity measurements using the needle probe method⁹ were made on sediment samples recovered in piston cores at each site and a single mean conductivity determined for each area. The mean heat flow at each site, weighted according to the errors in individual heat flow measurements, is given in Table 1 and compared with the normal heat flow expected for crust of this age in Fig. 2.

The heat flow measurements at each site are remarkably uniform. The 95% confidence limits on the weighted site means are $\pm 4 \text{ mW m}^{-2}$ (0.1 h.f.u.) at all but one site. The near-normal heat flow on the youngest part of the swell precludes any significant reheating of the upper part of the lithosphere. However, the gradual increase in heat flow over the older part of the swell is consistent with reheating of the lower part of the lithosphere as it will take several million years for these relatively deep temperature changes to diffuse upwards. While part of the increase in heat flow between sites C and E is attributable to the ~10 Myr age offset across the Molokai fracture zone (Fig. 1), the heat flow at sites F, G and H is still 7–12 mW m^{-2} higher than the expected heat flow for crust of this age. This anomaly, which is ~20–25% of the normal background heat flux, is clearly resolvable with the present data.

To determine if this heat flow anomaly is consistent with the amount of reheating required to uplift the swell, the expected heat flow for a simple reheating model has been calculated. The reheating process is assumed to begin at the lithosphere's base and proceed upwards, raising all lithospheric temperatures below a depth L to T_m , the assumed temperature of the asthenosphere. The initial temperature above depth L remains unchanged. While other forms of reheating are possible, this model puts the added heat as deep as possible and satisfies the constraint that no large heat flow anomaly is associated with the youngest part of the swell. In this model, the sea floor depth and heat flow at any point along the swell are completely determined by the geotherm (age) of the lithosphere when reheated, the initial height of the swell and the time since reheating⁸. If we assume an age of 90 Myr for the lithosphere around Hawaii and a plate model geotherm for the lithosphere¹⁰, then lithospheric thicknesses after reheating of 37–45 km are required to explain the observed swell uplift (corrected for the isostatic loading of

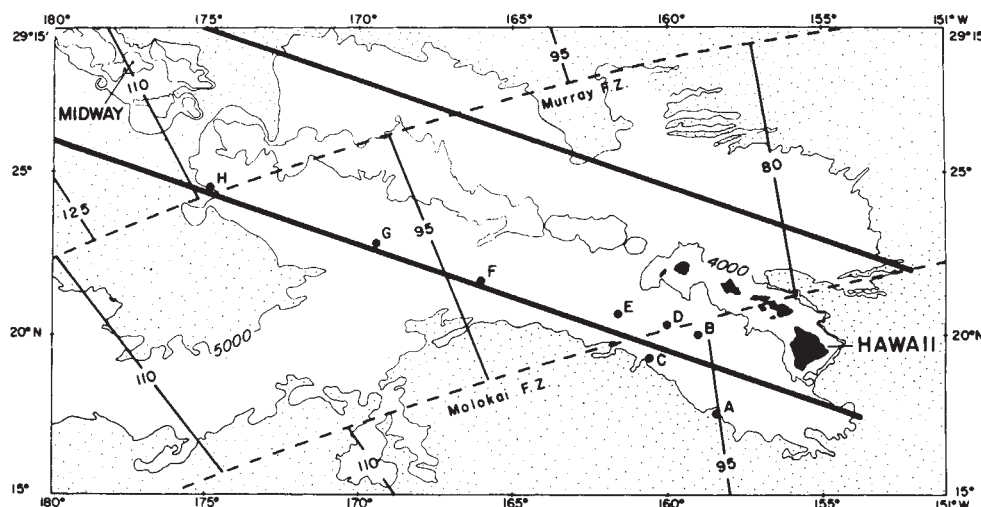


Fig. 1 Bathymetric map of the central Pacific with contours in metres. Isochrons (in Myr) and fracture zones (dashed lines) adapted from ref. 12. The extent of Hawaiian Swell is approximately indicated by the 5,000-m contour. ●, Heat flow site locations. Average depths along thick, black lines are plotted in Fig. 3.

Table 1 Summary of heat flow data on the Hawaiian Swell

Site	Location	N	$\bar{K}$ (Wm ⁻¹ K ⁻¹)	$\bar{Q}$ (mW m ⁻²)	s.d.	95% confidence limits (mW m ⁻²)	t_c (Myr)	t_H (Myr)
A	17°40' N 158°20' W	11	0.73	52.9	3.3	±2.3	95	2.1
B	20°00' N 159°00' W	8	0.77	51.5	2.0	±1.8	96	3.8
C	19°20' N 160°25' W	18	0.77	54.5	3.5	±1.8	98	5.0
D	20°15' N 160°02' W	4	0.74	58.7	3.4	±6.2	86?	5.0
E	20°30' N 161°30' W	8	0.81	58.0	2.9	±2.6	88	6.8
F	21°45' N 166°00' W	10	0.86	57.4	4.5	±3.4	94	12.1
G	22°56' N 169°36' W	18	0.95	58.3	4.0	±2.1	97	16.3
H	24°41' N 174°38' W	18	0.96	58.8	4.5	±2.3	109?	22.5

N , number of gradient measurements. $\bar{K}$, mean site thermal conductivity (corrected to *in situ* conditions). $\bar{Q}$, weighted mean heat flow with sample standard deviation s.d. t_c , Estimated crustal age. t_H , Inferred time since reheating. Crustal ages were estimated using the isochron map in ref. 12. Time since reheating was determined by projecting the site locations onto a line joining Hawaii and Midway and assuming an age of 27 Myr for Midway¹³.

100–200 m of sediment). These lithospheric thicknesses are about half the normal thickness of lithosphere of this age.

The predicted subsidence and anomalous heat flow for this range of values are shown in Fig. 3. The upper subsidence curve ($L = 37$ km) matches the data around Hawaii reasonably well, but seems too shallow over most of the rest of the swell. Inspection of the detailed bathymetry around the Hawaiian Islands¹¹ shows that there are two high spots on the swell, at 3 and 17 Myr, where smaller island chains (fracture zones?) crosscut the swell. Thus, the depths in these two areas may be anomalously shallow. The lower subsidence curve ($L = 45$ km) is a better fit over most of the swell. The heat flow anomaly observed on the swell also seems to be closer to the anomaly predicted by this lower subsidence curve, although the heat flow at the three oldest sites is less than predicted. This part of the swell actually formed when the hotspot was reheating slightly younger lithosphere (~80-Myr old) before it crossed the Molokai fracture zone (Fig. 1). When this is considered (dashed line in Fig. 3), the heat flow anomaly on this part of the Hawaiian Swell is probably large enough to be consistent with the observed swell uplift.

The main uncertainty in the present study is that reliable measurements of heat flow were not obtained on crust of the same age located off the swell, so the observed heat flow anomaly cannot easily be separated from any regional anomaly unrelated to the swell. The size of the observed anomaly is also small and close to the resolution of the data. Thus, although the

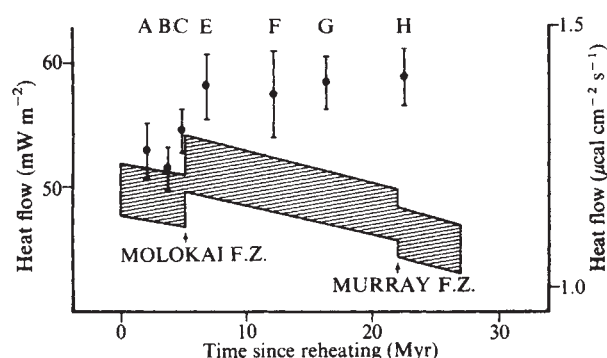


Fig. 2 Observed heat flow on the Hawaiian Swell with 95% confidence limits plotted against the time since each site passed over the Hawaiian hotspot. The shaded band is the normal heat flow expected for crust of this age estimated using a simple (age)^{-1/2} relation with a scale factor of 11–12 h.f.u. (ref. 10). Assumed crustal ages and reheating times are given in Table 1. Site D was omitted from this plot because the heat flow is poorly determined (only four measurements) and the site is probably located on the Molokai fracture zone.

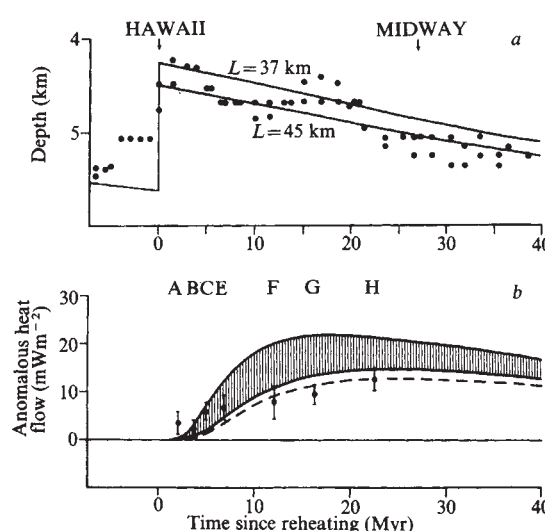


Fig. 3 *a*, Observed and predicted swell subsidence. Dots are 1° × 1° averages of the depth of the crest of the Hawaiian Swell along the lines shown in Fig. 1. Solid curves are predicted subsidence for reheating model where L is the lithospheric thickness after reheating. *b*, Anomalous heat flow observed on the Hawaiian Swell compared with predicted heat flow for $L = 37$ km (upper solid curve) and $L = 45$ km (lower solid curve) assuming an age of 90 Myr before reheating. These curves represent the upper and lower bounds on the heat flow anomaly required to explain the observed swell uplift. The dashed line shows the effect on the lower bound of reheating 80-Myr old rather than 90-Myr lithosphere (see text for discussion).

present measurements are consistent with lithospheric reheating, the strongest evidence for this model remains the manner in which the swell subsides.

This work was sponsored by NSF grant OCE79-19226 to the Woods Hole Oceanographic Institution. We thank Barry Parsons for helpful comments.

Received 27 January; accepted 30 April 1981.

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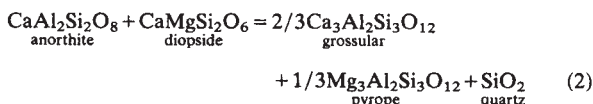
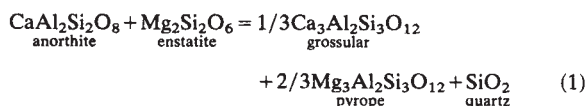
Charnockite geobarometers based on coexisting garnet–pyroxene–plagioclase–quartz

D. Perkins III & R. C. Newton

Department of the Geophysical Sciences, University of Chicago, Chicago, Illinois 60637, USA

Numerous geothermometers based on compositions of coexisting minerals have been calibrated experimentally or theoretically during the past 10 yr. In contrast, few continuous-reaction geobarometers exist which have had wide application. One, based on the reaction of cordierite to garnet, sillimanite and quartz, is limited by a relatively narrow pressure range of application and also by conflicting calibrations^{1,2} because the mixing relations of Mg and Fe and the role of H₂O in cordierite³ are not understood. Another successful barometer uses the assemblage garnet, plagioclase, Al₂SiO₅ and quartz^{4,5}. Continuous geobarometers based on the reaction of orthoferrosillite to fayalite plus quartz⁶ and on the aluminium content of enstatite in equilibrium with garnet⁷ have been used to a lesser extent. The former barometer is limited by rarity of the requisite assemblage, and the latter by uncertain experimental calibration for crustal rocks. We calibrate here two mineralogical geobarometers from measured thermodynamic data. The participating minerals, pyroxenes, garnet, plagioclase and quartz, are frequently associated in high-grade metamorphic rocks. Calculation of pressures for several terrains show that the geobarometers yield reasonable and consistent results for the entire range of crustal pressures.

Most high-grade metamorphic terrains are in Precambrian shield areas and are dominated by orthopyroxene-bearing (charnockitic) acid to intermediate gneisses which invariably contain abundant basic segregations and lenses (basic granulites). The assemblage pyroxene–plagioclase–quartz is essential to charnockitic rocks, and garnet is present in many, including the type Madras charnockite occurrence⁸. Garnet–pyroxene–plagioclase, frequently with quartz, is also characteristic of the basic units in many terrains. The four phase assemblages are represented by the reactions:



Because these reactions have large volume changes they are suitable for geobarometry. The reactions are of high variance in rocks: plagioclase contains large amounts of NaAlSi₃O₈ (albite) in solid solution; the garnets and pyroxenes of granulites are complex, containing major amounts of Fe and smaller amounts of other elements. Previous attempts to calibrate these barometers^{9,10} have used experimental syntheses of the four-phase assemblages in simple systems^{11,12}, but long extrapolations are necessary from experimental temperatures and compositions of the phases were not reversed. An earlier thermodynamic calibration of reaction (1) was hampered by incomplete data for the phases¹³. Data are now complete enough for relatively rigorous formulations of the geobarometers from thermodynamic measurements.

The equilibrium condition that the Gibbs energy change is zero for reaction (1) at given temperature, *T*, and pressure, *P*, of metamorphism leads to the following expression:

$$-\Delta H^\circ + T\Delta S^\circ \cong RT \ln \frac{(\alpha_{\text{Mg}}^{\text{Gt}})^2 \cdot (\alpha_{\text{Ca}}^{\text{Gt}})}{\alpha_{\text{En}}^{\text{Opx}} \cdot \alpha_{\text{An}}^{\text{Pl}}} + P\Delta V^\circ \quad (3)$$

where ΔH° and ΔS° are, respectively, the enthalpy and entropy changes of the reaction, ΔV° the molar volume change at *T*, and the α s denote the activities of MgAl_{2/3}SiO₄ and CaAl_{2/3}SiO₄ in garnet, Mg₂Si₂O₆ in orthopyroxene and CaAl₂Si₂O₈ in plagioclase. The approximation sign is used because of neglect of differential compressibilities and expansivities. A similar equilibrium condition is obtained for reaction (2).

Several recent thermochemical measurements allow evaluation of the left-hand side of equation (3), the standard Gibbs energy change, with moderate accuracy, thus avoiding the uncertain derivation from phase equilibrium studies. Enthalpy of solution measurements at 970 K for enstatite and pyrope¹⁴, diopside and grossular¹⁵ and anorthite¹⁶ give ΔH° as the difference in enthalpies of formation from the oxides. Low temperature adiabatic heat capacity measurements and high temperature heat content measurements, give ΔS° (refs 17–19). An entropy increment of 1.0 cal K⁻¹ is added to anorthite to account for Al, Si disorder²⁰. Over a temperature range of a few hundred degrees centred around 970 K, ΔH° and ΔS° are virtually constant.

The activities of Ca–Mg–Fe garnet components can be referred to constant *W* parameters for the bounding binary joins, such that the activity coefficient of MgAl_{2/3}SiO₄, $\gamma_{\text{py}} = \alpha_{\text{py}}/X_{\text{py}}$ is given by:

$$Rt \ln \gamma_{\text{py}} \cong W_{\text{MgFe}} X_{\text{Fe}}^2 + W_{\text{MgCa}} X_{\text{Ca}}^2 + (W_{\text{MgFe}} + W_{\text{MgCa}} - W_{\text{CaFe}}) X_{\text{Ca}} X_{\text{Fe}} \quad (4)$$

with a similar expression for γ_{gr} (ref. 21). W_{MgCa} has been measured calorimetrically and, for the ranges of compositions considered here, is, to a good approximation, W_{MgCa} (cal) = 3,300 – 1.5 *T* (K)⁴. The simplest values of W_{CaFe} and W_{MgFe} compatible with high temperature and high pressure phase equilibrium measurements are zero^{22,23}. The unknown mixing properties of Mn in garnets restricts consideration to garnets with Mn ≤ Mg/3. Enthalpy of solution studies on plagioclase¹⁶ lead to the 'Al-avoidance' activity model, expressed by

$$\alpha_{\text{an}} = \gamma_{\text{an}} \frac{X_{\text{an}}(1 + X_{\text{an}})^2}{4} \quad RT \ln \gamma_{\text{an}} = (1 - X_{\text{an}})^2(2,075 + 9,318 X_{\text{an}}) \quad (5)$$

Finally, the Mg₂Si₂O₆ and CaMgSi₂O₆ activities in pyroxene are taken as 'ideal two-site'

$$\alpha_{\text{en}}^{\text{Opx}} = X_{\text{Mg}}^{\text{M2}} \cdot X_{\text{Mg}}^{\text{M1}} \quad \alpha_{\text{di}}^{\text{Cpx}} = X_{\text{Ca}}^{\text{M2}} \cdot X_{\text{Mg}}^{\text{M1}} \quad (6)$$

where M1 and M2 are the pyroxene structural sites for non-tetrahedral cations. Following Wood and Banno²⁴, Al^{VI}, Cr, Ti and Fe³⁺ are ordered in M1, Ca, Mn and Na are ordered in M2, and Mg and Fe²⁺ are equipartitioned in M1 and M2.

The above considerations lead to the following geobarometric expressions for reactions (1) and (2), respectively, with *P* in bar and *T* in K

$$P = 3,944 + 13.070T + 3.5038T \ln \frac{(\alpha_{\text{Ca}}^{\text{Gt}}) \cdot (\alpha_{\text{Mg}}^{\text{Gt}})^2}{\alpha_{\text{En}}^{\text{Opx}} \cdot \alpha_{\text{An}}^{\text{Pl}}} \quad (7)$$

$$P = 675 + 17.179T + 3.5962T \ln \frac{(\alpha_{\text{Ca}}^{\text{Gt}})^2 \cdot (\alpha_{\text{Mg}}^{\text{Gt}})}{\alpha_{\text{Di}}^{\text{Cpx}} \cdot \alpha_{\text{An}}^{\text{Pl}}} \quad (8)$$

The calculated pressures depend on mineralogical determination of *T* with its inherent uncertainties, but, fortunately, expressions (7) and (8) are not sensitively dependent on *T*. The major source of uncertainty is the thermochemically measured standard Gibbs energies, which create pressure uncertainties of 1,400 bar for the orthopyroxene barometer and 1,700 for the clinopyroxene barometer at a given temperature. The precision of the barometers is better than this, as the ΔG° errors are nearly

Table 1 Sample data and average calculated pressures

Locality	Description	Sample (No. of samples)	Ref.	Estimated temperature (°C)	P (opx) (bar)	P (cpx) (bar)
Huntley-Portsoy, Scotland	Migmatite restite, aureoles	10,557 (1)	25	690	3,849	
Lochnagar, Scotland	of Newer Intrusives	82,891 (1)		780	2,845	
Nain, Labrador	Granulites, anorthosite	NAK2893, 3909 (4)	39	750–800	3,299	
New York	aureole					
Adirondack Highlands,	Charnockites and	ADAS-3 to	40	725–775	8,476	6,574
	metagabbros	ADTL-4 (12)				
Nilgiri Hills, India	Charnockites and mafic	11-1, 11-3 (2)	Unpublished	825	8,723	6,167
Biligirirangan Hills, India	granulites from South	7-5 (1)	Chicago	800	9,493	6,530
Sittampundi Complex, India	Indian shield	SITM-92 (1)	data	830		7,993
Madras, India		MP44, 72 (2)	7	790	7,406	
Finnish Lapland		47-III (1)	41	740	7,384	5,166
Buksefjorden, Greenland	Late Archaean–early	174087, 102 (2)	10	800	7,537	5,026
Furua Complex, Tanzania	Proterozoic granulites	TANZ 2-91 (14)	29	800	10,689	8,350
Labwor, Uganda		AR51 (1)	42	950	8,087	
Doubtful Sound, New Zealand	Deep crustal mafic granulites	36453-68 (7)	28	750	12,602	10,971
Ronda Complex, Spain		R-208-A (1)	27	800		11,100
Lashaine Volcano, Tanzania	Exotic granulite nodules	BD-727, 728 (2)	Unpublished Chicago data	950		15,550

constant. Assessment of accuracy of the barometers is more difficult. Information on this problem may be obtained by calculation of the pressures from a wide variety of granulite localities for which published mineral analyses exist.

Table 1 lists the localities, original sample numbers and descriptive data for several well documented occurrences of assemblages (1) and/or (2), representative temperatures, and calculated pressures. Immediately evident is that the clinopyroxene barometer reads 1–3 kbar lower than the orthopyroxene barometer. This discrepancy is within the combined uncertainties in ΔG° for reactions (1) and (2), and the ΔG° s could be adjusted to bring the barometers into agreement, on average. However, the pressure discrepancies may be real, and result from a greater tendency of clinopyroxene than orthopyroxene to re-equilibrate with plagioclase during uplift to lower pressures. Some information on the closure problem is given by the following pressure calculations from various terranes.

The Huntley-Portsoy and Lochnagar occurrences are hypersthene-bearing migmatites in the aureoles of the Newer Intrusions, southern Scottish Highlands²⁵. Both are sillimanite-grade, superposed on regional andalusite-bearing terrains, which suggests that the pressures should be lower than the experimental Al_2SiO_5 triple point. The Nain, Labrador granulites are in a high temperature aureole around a large anorthosite body. Pressure calculations based on the orthoferrosilite–fayalite–quartz reaction²⁶ give an average 3.2 kbar, in very good agreement with the present calculation. Buksefjorden, Greenland, Sittampundi Complex, Madras, Biligirirangan Hills and Nilgiri Hills, South India and Adirondack Highlands, New York, localities are all in large granulite tracts where sillimanite is the regional Al_2SiO_5 polymorph of intercalated metapelites. The orthopyroxene pressures agree with the Al_2SiO_5 diagram (Fig. 1) and seem more appropriate than previous pressure estimates, which would place the Adirondack⁶, Madras⁷ and Buksefjorden¹⁰ localities within the kyanite field. The Adirondack orthopyroxene pressure of $8,476 \pm 1,076$ bar based on 12 samples agrees with the current assessment²⁷ of 8 ± 1 kbar based on revised orthoferrosilite–fayalite–quartz barometry. Kyanite has been reported from the south-central Adirondacks²⁸, suggesting pressures close to the kyanite–sillimanite boundary. The Furua, Tanzania, granulite complex is a kyanite-bearing terrain²⁹, and the high calculated

orthopyroxene pressure is consistent with the experimental Al_2SiO_5 diagram (Fig. 1).

The Ronda aureole, southern Spain, and Doubtful Sound, New Zealand, granulites are tectonically uplifted portions of the deep crust^{30,31}. The Ronda granulites show a primary recrystallization at 11,100 bar followed by a secondary (corona) recrystallization at 6,480 bar. These estimates correspond closely to Obata's³² pressure estimates of two discrete recrystallizations of the Ronda peridotite mass. The Doubtful Sound mafic granulites are known, from gravity surveys, to be underlain by the upper mantle at a depth <10 km. At a distance of only 20 km inland, the New Zealand crust has a normal 40 km thickness. An average 2.8 g cm^{-3} crustal density and a 30–40 km uplift of the Doubtful Sound area implies pressures of 8.4–11.2 kbar of crystallization of the rocks now exposed at the surface.

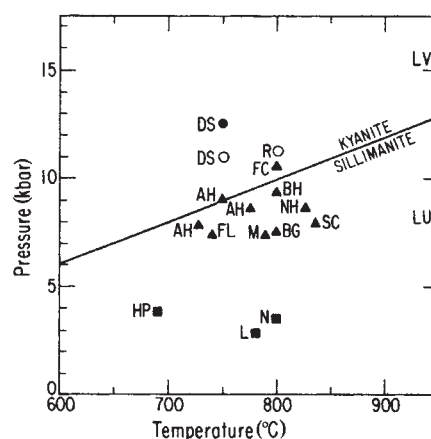


Fig. 1 Pressure calculations for various granulites. ■, Thermal aureoles (HP, Huntley-Portsoy; L, Lochnagar; N, Nain). ▲, Precambrian granulite terrains (AH, Adirondack Highlands; FC, Furua Complex; FL, Finnish Lapland; M, Madras; BH, Biligirirangan Hills; NH, Nilgiri Hills; BG, Buksefjorden; SC, Sittampundi Complex; LU, Labwor, Uganda). ●, Deep crustal granulites (DS, Doubtful Sound; R, Ronda; LV, Lashaine Volcano). Filled symbols, orthopyroxene (reaction (1)) barometer. Open symbols, clinopyroxene (reaction (2)) barometer. Temperatures mostly from literature estimates (see Table 1).

The Lashaine Volcano, Tanzania exotic nodule suite contains clinopyroxene-plagioclase-garnet-scapolite granulites with occasional quartz, which may or may not be in textural equilibrium with the other minerals. The indicated clinopyroxene pressure of 15.5 kbar (Table 1) is an overestimate if quartz is not actually part of the primary equilibrium assemblage, but is in agreement with Irving's³³ experimental conclusion that very similar nodules from Delegate, Australia, equilibrated with their source at 14–17 kbar. Such scapolite granulites may be prominent components of the lower crust³⁴.

Everything that is known about the crystallization pressures of the occurrences tabulated here seems to agree with the orthopyroxene (reaction (1)) geobarometer. The strongest indications of its validity are comparison with the experimental Al_2SiO_5 diagram and the orthoferrosilite-fayalite-quartz geobarometer. This evidence suggests that reequilibration to lower pressures after the peak of metamorphism has had minimal effect on the pressure indication of reaction (1). The two barometers can be made to agree on the average within the uncertainties of the thermochemical measurements by a balanced readjustment of the ΔH° s and ΔS° s leading to the following expressions:

$$P(\text{opx}) = 3,694 + 12.820T + 3.5038T \ln K_1$$

$$P(\text{cpx}) = 1,425 + 17.929T + 3.5962T \ln K_2 \quad (9)$$

These readjustments give the maximum allowable increase in the clinopyroxene pressure with only a small decrease in the orthopyroxene pressure. A test of the modified clinopyroxene barometer and some information on the delayed closure problem may be possible using lower-crustal two-pyroxene garnet granulite samples from explosive pipes³⁵, which are effectively quenched from deep-seated conditions. We have not yet found sufficient published data of this kind for a definitive test.

A firm result of this study is that the continental crust was, locally, at least, of thickness approaching or exceeding that of the present normal thickness as long ago as the late Archaean. A proposed high-temperature, low-pressure charnockite geotherm³⁶ is not supported. The remarkable cluster of Precambrian granulite pressures (Fig. 1) at 8.5 ± 2 kbar (opx barometer) for nine different terrains suggests that a certain repeated mechanism of high grade metamorphism has been operative. For the western Grenville this mechanism may have been continental collision, as the abortive rift of the North American continent which began ~1,400 Myr ago, closed ~1,100 Myr ago, generating the Grenville orogeny. The Grenville supracrustals were buried at 30 km depth in a doubled continent³⁷. Continental collision with temporary doubling of the crust has been proposed to explain the apparently high pressures of late Archaean metamorphism also³⁸. Great uplift in post-Precambrian times exposing deep crust and even upper mantle would seem to be required by the high pressures of the Doubtful Sound and Ronda occurrences.

We thank J. N. Berg, S. R. Bohlen, E. C. Hansen and A. S. Janardhan for unpublished mineral analyses, and W. L. Griffin for helpful comments. This research was supported by NSF grant EAR 78-15939 (R.C.N.). Some of the Indian granulites described here were gathered and analysed under an NSF grant, EAR 79-05723 (R.C.N.).

Received 23 February; accepted 8 May 1981.

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Preservation of polyunsaturated fatty acid in ancient Anasazi maize seed

David A. Priestley*, Walton C. Galinat† & A. Carl Leopold*‡

* Boyce Thompson Institute, Ithaca, New York 14853, USA

† University of Massachusetts, Suburban Experiment Station, Waltham, Massachusetts 02154, USA

Oxidation of polyunsaturated fatty acids (PUFAs) is widespread in animal tissues and has been implicated in senescence¹ and as a cause of deterioration of stored seeds². Although signs of lipid oxidation have been detected in seeds during long-term storage³, little is known of the magnitude of the change induced in the PUFA fraction. In conditions of 'accelerated ageing' (saturating humidity and 45 °C) in soybean seeds, the PUFA content has been reported to decline within a few days⁴, but in somewhat milder conditions PUFAs are not oxidized during the time when germinability is lost⁵. An extreme case in which to estimate the extent to which PUFAs may be oxidized comes from the examination of ancient maize seeds from caves in the southwestern United States, where the arid climate is conducive to the preservation of biological materials⁶. We have now compared new seeds with seeds which were 1–17 centuries old, and report that most of the linoleic acid (by far the predominant PUFA in this species) is oxidized in the first 100–200 yr, but some is still detectable in seeds more than 1,500 yr old. We believe this represents the oldest sample of PUFA so far reported.

Modern maize seeds (*Zea mays* L.) contain ~5% lipid, most of which is neutral storage lipid. Four principal fatty acids are found (C18:2 > C18:1 > C16:0 > C18:0). In a representative modern North American variety about half of the fatty acid is linoleic, C18:2 (Table 1; cv. Early Cornell Synthetic). The polar lipids of these seeds display a fatty acid profile rather similar to that of the storage lipid fraction. Increasing evidence indicates that maize has evolved through several thousand years of artificial selection from the annual teosinte (*Z. mexicana* (Schröder) Kuntze)^{7,8}. In agreement with an earlier study⁹, we

‡ To whom correspondence should be addressed.

Table 1 Total fatty acid content of maize and teosinte seeds

a, Recently grown seeds		% Of total				mg Fatty acids per g seed material
Sample		Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	
<i>Z. mays</i> (cv. Early Cornell)		12.7	2.2	30.6	54.5	38.2
Synthetic		(0.2)	(0.1)	((.1)	(0.2)	(0.2)
<i>Z. mexicana</i> (race Nobogamé)		15.5	2.2	38.2	44.1	6.3
		(0.2)	(0)	(0.1)	(0.1)	(0.1)
<i>Z. diploperennis</i>		14.6	1.9	37.0	46.5	13.5
		(0.2)	(0)	(0)	(0.2)	(0.2)
b, Old seeds		% Of total				mg Fatty acids per g seed material
Sample no.	Mean of estimated age (yr)	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	
1	1,700	35.3	8.7	51.3	4.8	11.4
		(0.5)	(0.2)	(0.1)	(0.3)	(0.3)
2	1,700	35.1	7.6	50.0	7.4	16.1
		(0.3)	(0.1)	(0.5)	(0.3)	(0.9)
3	1,500	36.0	9.2	50.9	4.0	14.5
		(0.5)	(0.8)	(2.7)	(1.5)	(0.9)
4	1,000	35.0	7.4	50.5	7.1	5.6
		(0.8)	(0.8)	(0.2)	(0.4)	(0.5)
5	900	56.0	9.2	34.9	—	7.3
		(1.2)	(0.3)	(0.8)		(0.7)
6	97	35.3	6.9	46.4	11.5	13.4
		(0.2)	(1.9)	(0.1)	(1.9)	(1.7)
7	78	18.2	3.9	44.2	33.8	21.9
		(0.2)	(0.5)	(0.2)	(0.5)	(0.9)

The lipids of ground seed particles (0.8–1.6 g) were extracted with chloroform-methanol (2:1, v/v) and the fatty acids prepared for gas chromatography as previously described³. Separations were performed either on a 1.8×4 mm internal diameter glass column packed with 3% Silar-5CP on 100/120-mesh Gas Chrom Q or a 1.8×2 mm glass column packed with 10% DEGS-PS on 80/100-mesh Supelcoport. Additional details are given in ref. 5. Values given are the mean (and range) of two injections. Traces of linolenic acid (C18:3) in recent samples have been ignored.

Table 2 Provenance of old maize seeds

Sample no.	Race or variety	Date AD (and cultural affiliation)	Location	Ref.
1	Evolved Chapalote	100–500 (Basketmaker)	Cave II, March Pass, Kayenta, Arizona (PM: A 2481)	12
2	Early Chapalote	100–500 (Basketmaker)	As above (PM: A 2481)	12
3	Near Chapalote	200–700 (Basketmaker)	Site SR 16–6, Dirty Devil-Waterhole Flat, Utah (PM: 10/264)	24
4	Near Maíz de Ocho	700–1300 (Kayenta Anasazi)	Cave 8, Sagiotsosi Canyon, Kayenta, Arizona (PM: A 3520)	12
5	Dented Chapalote	950–1200 (Fremont)	Site PR4-31, Nine Mile Canyon, Utah (PM: A 7769)	24
6	Florida White Dent	1883	Central Kentucky (WCG)	
7	Leaming Dent	1902	Agricultural Expt. Station, Urbana, Illinois (WCG)	

The age ranges given for the ancient seeds are derived from the stratigraphy of the sites and are consistent with the overall pattern of maize evolution. Further details are given in ref. 12. PM, Catalogue number of the Peabody Museum, Harvard University; WCG, private collection of Dr Walton C. Galinat.

found that contemporary seeds of annual teosinte contain less lipid than maize. The proportions of the fatty acids are similar for maize, annual teosinte and a recently discovered perennial teosinte (*Z. diploperennis* Iltis, Doebley and Guzmán)¹⁰ (Table 1). In each of these contemporary seeds, linoleic acid constitutes >40% of the total fatty acids present¹¹. It is likely that the original content of linoleic acid in the ancient maize seed would have matched or exceeded this value.

Maize has been associated with the Anasazi culture of the American South-West from the first appearance of its earliest representatives (the Basketmaker people) around AD 100. This association continued throughout the Classic Pueblo period and into the recent era of European influence. The changing morphology of the maize races during this long period has been studied in some detail¹². We examined four samples of maize from various periods of the Anasazi culture (Tables 1 and 2, samples 1–4) and one sample from the closely related Fremont

culture (sample 5). We also investigated maize seed grown during the past 100 yr (samples 6 and 7). The provenance of the seeds is detailed in Table 2. The ages assigned to the seeds are consistent with the stratigraphy of the sites from which they were obtained and with the overall pattern of maize evolution¹². All these seeds were non-viable. When received for analysis their water content was ≤5–6%. All the ancient seeds showed excellent external preservation, and gave a light or dark brown meal when ground. The single Fremont sample (no. 5) was unusual in having adhering soil particles (presumably indicative of a moist environment) and in being extremely friable, readily crumbling into a fine dust when ground.

With the exception of the Fremont seed, all the samples contained linoleic acid (Table 1). The relative proportion of the three other fatty acids followed the customary maize-teosinte pattern (C18:1>C16:0>C18:0). Similar results were obtained whether we used a cyanosilicone phase or a polyester

phase for the gas chromatographic analysis. The presence of the C18:2 in the oldest seeds has been further confirmed by combined gas chromatography-mass spectrometry (data not shown). Results were comparable with those of Table 1 when the fatty acids of the polar (presumably primarily membrane) lipid subfraction were examined (data not shown). The PUFAs of the storage lipid fraction and the membrane lipid fraction were therefore about equally susceptible to oxidation. The data of Table 1 suggest a relatively rapid decline in linoleic acid during the first 100–200 yr of storage, leaving a small proportion of linoleic acid which is comparatively stable for much longer.

The proportion of palmitic acid (C16:0) in the Fremont sample exceeded that of oleic acid (C18:1). In these badly preserved seeds, even the monoenoic fatty acid was probably subject to enhanced degradation compared with the fully saturated acids. The well known deleterious effects of damp soil on ancient seeds¹³ probably led to loss of oleic acid in the Fremont seeds. On the other hand, prolonged dry storage of the well preserved Anasazi seeds (samples 1–4) was sufficient to remove only ~80% of the linoleic acid and scarcely any of the oleic.

Although preservation of monoenoic fatty acids has been noted in well stored Roman oil samples from the second century AD^{14,15} and brain lipids of a 40,000-yr-old frozen mammoth¹⁶, a badly deteriorated Egyptian oil sample originating from the sixth century BC lacked all but the fully saturated fatty acids¹⁷. PUFAs were absent in each case.

It is unlikely that the linoleic acid in the Anasazi seeds represents contamination, microbial or otherwise, because both the total (primarily neutral) lipid fraction and the quantitatively minor polar (membrane) lipid subfraction yielded similar results and it is most unlikely that these two different fractions would be contaminated to approximately the same extent in every sample.

The long-term preservation of linoleic acid is consistent with reports that some membrane structure can be preserved in ancient seeds, which suggests the presence of sufficient membrane lipid to permit a bilayer structure. Both membrane profiles in Egyptian wheat seeds from the third and fifth millennia BC¹⁸ and ample membrane preservation in 'fossil' Indian lotus seeds¹⁹ (exact age uncertain)²⁰ have been reported. The widespread occurrence of tocopherols in seeds²¹ doubtless serves to minimize the prevalence of PUFA oxidation during storage.

Our data refer to changes which occur in maize seeds post mortem, and do not relate directly to the debate about the relevance of lipid peroxidation to loss of seed viability^{4,5,22,23}. However, they do indicate that the extensive PUFA oxidation which may occur in seeds during a few days or weeks of 'accelerated ageing' in the laboratory may take many decades or centuries in drier and cooler conditions. Furthermore, the retention of significant amounts of linoleic acid after 1,500 yr or more of storage emphasizes the unusual capacity of dry seeds to protect at least part of their susceptible components from atmospheric oxidation.

We thank Dr H. Everett and Bill Tracy of Cornell University for recent seed of maize and perennial teosinte, and Dr Alan Renwick for gas chromatography-mass spectrometry analysis.

Received 10 November 1980; accepted 8 April 1981.

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Lack of correlation between extracellular polysaccharide and nodulation ability in *Rhizobium*

Richard Sanders*†, Elisabeth Raleigh† & Ethan Signer†

* Department of Chemistry, University of Colorado, Boulder, Colorado 80309, USA

† Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Rhizobia are Gram-negative bacteria normally capable of nodulating the roots of leguminous plants, and the failure of five EPS (extracellular polysaccharide)-deficient mutants to nodulate suggested that EPS is required for this nodulation. However, we report here that among a larger sample of mutants with altered EPS production, isolated from two species of *Rhizobium* (including one of the original set plus 34 new ones), production of EPS is not correlated with ability to nodulate appropriate hosts. Therefore, although involvement of a minor EPS component cannot be ruled out, there is no evidence that gross EPS is required for nodulation.

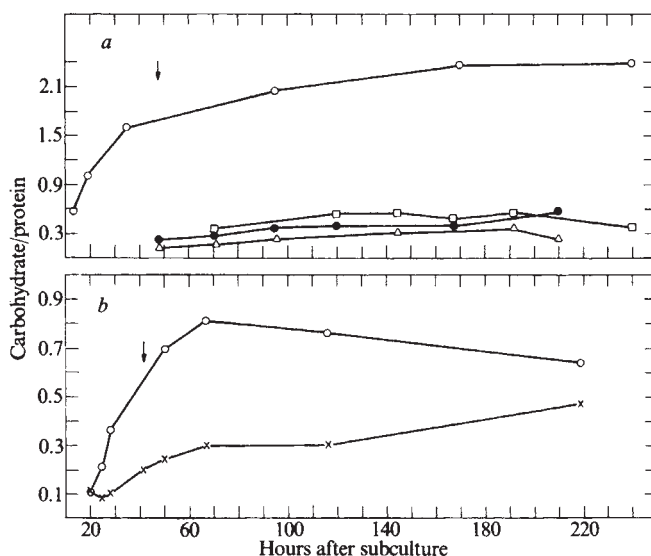


Fig. 1 *a*, Bacteria were grown in MBM⁴; 1 ml of a fresh stationary-phase culture was inoculated into 100 ml of fresh medium and shaken at 30 °C, and a 10-ml sample was taken at each time point. Arrows indicate the onset of stationary phase. Aliquots (5 ml) of each sample were passed through a 0.45-μm Millipore filter and the filtrate dialysed against three changes of distilled water before being assayed for carbohydrate using the anthrone method⁵. The remaining 5 ml were dialysed as above and assayed for protein by the Lowry method⁸. Numbers are glucose-anthrone units per bovine serum albumin Lowry unit. Each point represents the average of a least three independent experiments. We have examined the residual anthrone-positive material made by *R. leguminosarum* 128c53 str' rif' RS1 and found that ~50% of it is excluded from Sepharose 4B, where essentially all wild-type material enters the column. This peak of aggregated or high-molecular-weight material is rich in 2-keto-3-deoxyoctanoic acid⁹, suggesting that it consists of cell wall fragments rather than extracellular material¹⁰. Therefore, low EPS values are likely to be overestimates, and we do not consider that the assay used here is accurate below 10% of wild-type production. ○, 128c53 str' rif'; ●, RS4; △, RS5; □, RS9. *b*, Bacteria were grown and assayed as described in *a*. Each point is the average of two independent determinations. ○, 127K26; ×, ER12.

Table 1 Extracellular polysaccharide production and nodulation ability of mutants isolated by *a*, the membrane-filter technique and *b*, phage selection

<i>a</i>			<i>b</i>		
Strain	% Wild-type EPS (100)	% Wild-type nodules (100)	Strain	% Wild-type EPS (100)	% Wild-type nodules (100)
128c53 <i>str rif</i>			127K26		
RS1	6.1	≤2	FB30	108	100
RS2	8.9	10±5	ER2	32	≥60
RS3	15.5	25±5	ER19	28	≥60
RS4	9.6	110±5	ER3	6.9	≥60
RS5	9.1	≤2	ER13	8.3	≥60
RS6	6.1	≤2	ER17	8.1	≥60
RS7	6.5	≤2	ER24	6.0	≥60
RS8	13.0	≤2	ER26	4.3	≥60
RS9	20.0	≤2	ER21	22	<0.2
RS10	7.4	≤2	ER23	23	<0.2
RS11	6.4	≤2	ER204	11	0.2
RS12	5.5	≤2	ER15	10	<0.2
RS13	12.6	≤2	ER6	4.7	6
RS14	6.5	≤2	ER8	3.4	<0.2
RS15	11.6	≤2	ER12	9.2	<0.2
RS16	13.8	≤2	ER14	6.4	<0.2
			ER16	12	<0.2
			ER203	5.8	<0.2
			FB308	8.9	<0.2
			ER1	36	<0.2
			ER20	202	<0.2

a, Strains were assayed for EPS at 96 h after a 1:100 dilution of a stationary-phase culture into fresh medium as described in Fig. 1 legend. All values represent the average of at least three independent experiments. For nodulation assays pea seeds were surface sterilized by stirring in 30% H₂O₂ for 60 min, planted in a sterile 1:1 mixture of perlite and vermiculite (Grace), and watered daily with sterile distilled water. *R. leguminosarum* 128c53 *str rif*⁺ and its derivatives (5 ml) were grown with shaking at 30 °C to stationary phase in modified Bergersen's medium (MBM)⁴, resuspended in 5 ml of sterile water and inoculated onto 10-day-old Early Market (Agway) pea seedlings. The seedlings were grown at 26 °C with a 14-h light/10-h dark cycle, illumination by 3,000 ft-candles, and collected at day 21. All values represent the average of at least three independent experiments, each of which contained at least three plants. In each experiment at least three seedlings were inoculated with *R. leguminosarum* 128c53 *str rif* as positive control. Negative controls for each experiment were several uninoculated plants equal to the number of experimentally inoculated seedlings. Experiments in which the negative controls contained any nodules were discarded. Nodules were surface sterilized by stirring in 70% ethanol for 5 min, crushed, resuspended in sterile water, and spread on plates containing MBM for inspection of colony morphology. From RS2, 3 and 4 we recovered only non-slime-forming colonies identical to the inoculum. From rare nodules formed on plants inoculated with RS1 and RS5-16 we occasionally recovered colonies, usually a mixture of wild (slime-forming) and mutant types but sometimes mutant only; the slime-formers nodulated with wild-type efficiency and are presumed to be revertants. We found rare nodules with RS1, in contrast to the initial study, possibly due to differences in cultivar or conditions of temperature and light. *b*, Strains which prefixed ER were isolated from 127K26; FB308 was isolated from FB30, a streptomycin-resistant derivative of 127K26 (E.R., in preparation). For convenience, mutants are grouped by classes, to be described elsewhere. Cultures were inoculated 1:500 into fresh medium and collected 48 h after stationary phase was attained. Values are the average of three independent experiments for all except FB30, ER1 and ER8, which are the average of two independent experiments. ER1, which cannot use glutamate, was grown in MBM + 0.2% (NH₄)₂SO₄; the value is in comparison with 127K26 grown in the same medium (which gave a value of 98% of that for MBM alone). For the nodulation assays, five independent colonies of each mutant strain, six of FB30 and seven of 127K26 were grown in YAP (0.1% yeast extract, 0.1% L-arabinose, 0.5 × 10⁻⁴ M Na₂HPO₄, 6.65 × 10⁻³ M KH₂PO₄, pH 6.6)⁵ with 5 mM MgSO₄ added, for 3 days. Plants were inoculated with ~5 × 10⁸ bacteria for each culture. Each culture was tested at the time of inoculation by cross-streaking against five typing phages. Plants were prepared for the nodulation assay according to Beringer⁶ except that Fahraeus medium⁷ containing 1% agar was used. Seedlings were grown as described in *a*. Nodulation was monitored every 2-3 days by inspection for 15-20 days, at which time the plants were collected. Nodules began to appear on day 6; all strains recorded as ≥60% for nodulation had produced red nodules by day 12, and gave positive acetylene reduction assays for excised nodules at the time of collection. Plants inoculated with wild-type 127K26 had 50-150 nodules (average ~100) in various stages of development at the time of collection. Nodules were counted individually, up to 70, and the number remaining was estimated by eye. No nodules were found for any strain listed as <0.2. ER204 yielded one white nodule, among the five plants inoculated, which contained bacteria like the inoculum; two plants inoculated with ER6 yielded a total of 28 nodules which contained only revertant bacteria, as judged by colony morphology and phage type. A plant inoculated with YAP only had no nodules.

RS1 of *Rhizobium leguminosarum* 128c53 *str rif*⁺ is EXO-1 of ref. 1; the remaining mutants of this strain were isolated by the membrane-filter technique previously described¹. EPS-deficient derivatives of *Rhizobium phaseoli* 127K26 were found among survivors of exposure to bacteriophage F1, isolated from soil².

Figure 1 shows the kinetics of EPS production after subculture (see Fig. 1 legend) for *R. leguminosarum* 128c53 *str rif*⁺, *R. phaseoli* 127K26 and several of their EPS-deficient mutants. Table 1 compares EPS production, as measured by the anthrone method³, at 96 h after subculture (compare with Fig. 1), with nodulation ability for all mutants.

The main finding is that one mutant of *R. leguminosarum*, RS4, and five mutants of *R. phaseoli*, ER3, 13, 17, 24 and 26, make <10% of wild-type anthrone-positive material, yet nodulate normally. Other mutants have intermediate EPS and normal nodulation (ER2, 19), intermediate EPS and intermediate nodulation (RS3), intermediate EPS and little or no nodulation (RS8, 9, 13, 15, 16; ER1, 15, 16, 21, 23, 204), little EPS and poor nodulation (RS2), and even excess EPS and no nodulation (ER20).

Obviously, the crude methods used here do not distinguish between different carbohydrate components of EPS and our data would not reveal whether a minor component of EPS was

essential for nodulation. Nevertheless, it is clear that gross EPS production cannot be used as an indicator of nodulation ability and, therefore, that there is no evidence of a requirement for EPS in nodulation.

This work was supported by NSF grant PCM75-13897 and Rockefeller Foundation grant RFGAAS 7510 to Peter Albersheim and by PHS grant AI16873 from the NIH, NSF grant PCM77-19214, US Department of Agriculture grant 59-2253-0-1-498-0, and a grant (MV-38J) from the American Cancer Society to E.S. E.R. is supported by National Research Service Award GM07287. R.S. thanks the Helen Hay Whitney Foundation for financial support.

Received 13 February; accepted 31 March 1981.

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Do carbonate skeletons limit the rate of body growth?

A. Richard Palmer

Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9
Bamfield Marine Station, Bamfield, British Columbia, Canada V0R 1B0

Many marine invertebrates produce mineralized skeletons whose form suggests an economical construction. Porous as opposed to solid skeletal components are produced by scleractinian corals^{1,2}, balanoid and coronuloid barnacles³, ostreid and hippuritid bivalves⁴, and almost all modern echinoderm classes^{5,6}. They were also produced by rudist bivalves which were widely successful in Cretaceous tropical seas⁷. Marine prosobranch gastropods produce a stout external shell sculpture in place of a uniformly thick, ultimately stronger shell^{8,9}. The costs implied by these patterns of economical skeleton construction, however, are unknown¹⁰⁻¹². The term 'cost' refers to an evolutionary cost measured in terms of reduced fitness. Note that not all such costs are energetic; non-energetic constraints may also influence fitness. I present here evidence that both thick- and thin-shelled morphs of *Thais* (= *Nucella*) *lamellosa* (Gastropoda, Prosobranchia) produce shell material at a remarkably similar rate during maximal growth. Thick-shelled animals, however, exhibit a significantly slower rate of body growth. These results suggest that rates of skeletal growth can limit the rate of body growth and that this limitation represents a potentially important evolutionary cost.

Costs associated with carbonate skeletons can be subdivided into at least three categories: (1) an energetic, depositional cost including organic matrix production and mineralization; (2) an energetic, post-depositional cost, limited primarily to organisms that must expend energy transporting a skeleton; and (3) a non-energetic, growth-rate-limitation cost where the rate of maximal body growth may be set not by the energy available for growth but rather by the maximum rate at which skeleton may be produced. This latter cost is important to recognize because different patterns of energy consumption and growth will be exhibited with increasing availability of energy than would be expected if skeleton costs were strictly energetic.

Figure 1 illustrates the relationship between body growth and increasing energy (food) availability for three alternative hypotheses about factors which potentially limit body growth rate. Predictions about the relative rates of shell growth and rates of energy consumption for each hypothesis are included in Fig. 1 legend. The first hypothesis (Fig. 1a) predicts that if a physiologically determined upper limit to the rate of tissue production (dotted line) is reached before some upper limit to the rate of ingestion or assimilation (dashed line), rates of body growth in organisms with thick and thin skeletons will be identical when energy availability is high. Given an energetic cost to shell material, however, organisms with a thick or dense skeleton will require more energy to achieve the same rate of body growth because of additional energy expended in producing more skeletal material. The second hypothesis (Fig. 1b) predicts that if an ingestion (or assimilation) limit (dashed line) is reached before a tissue production limit (dotted line), then organisms with thick or dense skeletons will exhibit a lower maximal rate of body growth than those with thin or porous skeletons. Here, organisms with thick and thin skeletons should ingest (assimilate) equal amounts of energy at high energy availability, but because of energy invested in shell, thick-shelled animals should have a slower rate of body growth.

The third hypothesis (Fig. 1c) predicts that if a shell production limit is reached before either an ingestion or a tissue production limit, organisms with thick skeletons will again show slower rates of body growth at high energy availability, as with hypothesis b. However, because body growth cannot proceed in advance of skeleton growth, organisms with thick or dense skeletons should consume less energy (food) than those with thin or porous skeletons.

To test these hypotheses, pre-reproductive individuals of similar body weight (Table 1) were selected from a thick- and a thin-shelled population of the morphologically variable, intertidal gastropod, *Thais lamellosa*. Although having similar body weights, thick-shelled individuals had almost 50% heavier shells (Table 1). Growth experiments (Tables 1, 2) demonstrated that both thick- and thin-shelled animals added significantly less shell (mean, 22.6% less) and less tissue (mean, 25.2% less) at the upper than the lower tidal level. At both tidal levels, however, thick-shelled animals consumed fewer barnacles (Table 3) and also gained less body weight (mean, 32.5% less). Of particular interest is the fact that thick- and thin-shelled animals of the same initial body size produced almost equal weights of shell material at both tidal heights. These patterns of growth and prey consumption are inconsistent with the first two hypotheses (Fig. 1a, b), and support the skeleton-limitation hypothesis (Fig. 1c)

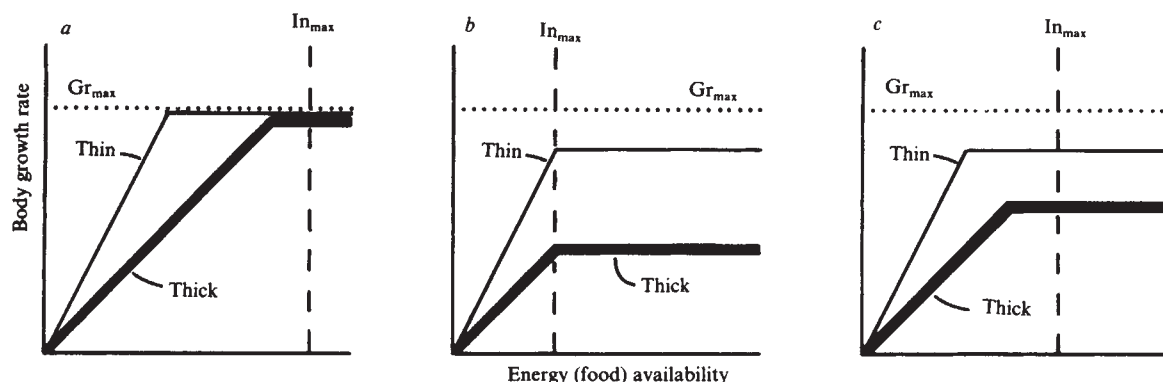


Fig. 1 Graphical illustration of hypothetical growth patterns with increasing rate of energy (food) supply for thick- and thin-shelled organisms subject to different growth constraints. *a*, Maximum rate of body growth limited by maximum rate of tissue production. *b*, Maximum rate of body growth determined by maximum rate of ingestion (or assimilation). *c*, Maximum rate of body growth determined by maximum rate of shell production. Predicted differences in rates of shell production at maximum body growth rate are: *a*, *b*, thick > thin; *c*, thick = thin. Expected energy (food) intakes at maximum body growth rate are: *a*, thick > thin; *b*, thick = thin; *c*, thick < thin. These differences will only be true when animals are growing at a maximum rate. In_{max} , potential maximum rate of ingestion (assimilation). Gr_{max} , potential maximum rate of body growth. Thin-lined curve, organism with low-density skeleton or thin shell; thick-lined curve, organism with high-density skeleton or thick shell.

Note for hypothesis *c* that the inflection points of the two curves need not coincide on either axis, in contrast to hypotheses *a* and *b*.

Table 1 Size and growth parameters for thick- and thin-shelled *T. lamellosa*

Tidal height (ft)	Morph	n	Initial			Total weight change	
			Shell length (mm)	Shell weight (g)	Body weight (g)	Shell weight (g)	Body weight (g)
0.0	Thick	10	21.9 (1.08)	1.68 (0.370)	0.23 (0.052)	1.43 (0.309)	0.37 (0.133)
	Thin	9	22.2 (1.26)	1.06 (0.169)	0.24 (0.042)	1.49 (0.177)	0.50 (0.092)
+2.0	Thick	14	21.6 (0.95)	1.59 (0.208)	0.22 (0.039)	1.06 (0.260)	0.25 (0.074)
	Thin	15	21.5 (1.67)	0.95 (0.216)	0.23 (0.045)	1.19 (0.329)	0.41 (0.138)

Values are means and standard deviations of size and growth parameters for thick- and thin-shelled *T. lamellosa* pooled from replicates at two tidal heights. Thick-shelled animals were collected from False Bay and thin-shelled animals from Turn Rock, both located on San Juan Island, Washington, USA. Shell and body weights were measured non-destructively by methods described elsewhere⁴⁰. Replicate groups of individually marked, measured and weighed animals of each shell type were placed in plastic screen cages mounted on pilings at two tidal heights (0.0 and +2.0 ft above MLLW⁴¹) at the Friday Harbor Laboratories, Friday Harbor, Washington, USA. Throughout the experiment the *Thais* were supplied freely with their preferred prey, *Balanus glandula*, on small stones⁴¹. Prey were made available at all times in high densities so that searching times were negligible. Snails were supplied with barnacles from 13 August to 5 September and 14 September to 14 October, and final body weights (wet) were measured on 13 November, 1978. *n*, Total number of individuals in each treatment. Numbers in parentheses are pooled standard deviations. There were two replicate cages for each treatment at the lower, and three for each treatment at the upper tidal level.

Table 2 Analysis of variance on initial values and two growth parameters of thick- and thin-shelled *T. lamellosa* at two tidal heights

Source of variation	d.f.	Initial				Growth parameter			
		Shell weight		Body weight		Shell weight change		Body weight change	
		MS	P	MS	P	MS	P	MS	P
Main effects									
Shell morph	1	5.101	0.001*	0.0002	0.737	0.118	0.244	0.283	0.001*
Tidal height	1	0.056	0.339	0.0013	0.419	0.880	0.002*	0.103	0.008*
Interaction	1	0.009	0.701	0.0001	0.844	0.014	0.686	0.001	0.857
Residual	44	0.063		0.0020		0.088		0.014	
Total	47	0.170		0.0021		0.112		0.022	

d.f., Degrees of freedom; MS, mean square; *P*, exact probability of *F* values obtained by dividing the component mean squares by the residual mean square. Data are from Table 1.

* Significant *F* ratios.

Table 3 Consumption rates of barnacles, *Balanus glandula*, by thick- and thin-shelled *T. lamellosa* at two tidal heights

Consumption rates			Two-way analysis of variance on consumption rates			
Tidal height (ft)	Shell morph		Source of variation	d.f.	MS	P
	Thick	Thin				
0.0	0.93	1.15	Main effects			
	1.01	1.28				
+2.0	0.56	0.85	Shell morph	1	0.120	0.029*
	0.63	1.02	Tidal height	1	0.231	0.007*
	0.85	0.78	Interaction	1	0.002	0.725
			Error	6	0.015	
			Total	9	0.049	

Consumption rates were measured as barnacles eaten per snail per day from 14 September to 14 October, 1978. Each value represents an average for five snails (individual consumption rates are not known as five snails were contained in each cage). An analysis of variance was performed on these average values: d.f., degrees of freedom; MS, mean square; *P*, exact probability.

* Significant *F* ratios.

that rate of shell production limits the maximum rate of body growth. However, these data cannot rule out the possibility that animals from the thick-shelled population consumed barnacles more slowly for other reasons and that the production of equal weights of shell material was coincidental. That this occurred at both tidal heights due to chance alone seems unlikely.

Two otherwise enigmatic patterns of invertebrate growth may be explained by the skeleton-limitation hypothesis. First, shell production continues in the absence of feeding in species of barnacles¹³, meso-¹⁴ and neogastropods (A.R.P., unpublished results), and both pteriomorphian^{15,16} and heterodont¹⁷ bivalves. Although difficult to explain energetically, this may reflect either an increase in a shell's habitable volume in anticipation of future body growth or a reinforcement of thinner shell produced during periods of more rapid growth¹⁸. Second,

scleractinian corals frequently show seasonal changes in the density of skeletal material produced¹⁹. Higher density bands are generally believed to form during periods of slower growth (refs 19–22). In both examples, energetic considerations would predict a decrease in skeleton production when tissue growth decreases or stops, yet calcification continues. Both examples would thus seem to be more readily explained as a response to skeleton-limited rates of body growth, rather than as a result of a high energetic cost to skeletal material.

A third growth pattern may also be explained by the skeleton-limitation hypothesis: rapidly growing organisms frequently produce less skeleton per unit body weight (for example, species of ramose scleractinian corals^{20,23}, pteriomorphian²⁴ and heterodont²⁵ bivalves, echinoids²⁶ and echinoderms in general²⁷). In addition, a number of organisms exhibit ontogenetic

changes in skeletal growth. Juveniles, for whom maximal growth may be critical^{9,28}, exhibit more porous or, in some cases, significantly less massive skeletons than adults (for example, species of balanoid and coronuloid barnacles^{29,30}, strombid and cypraeid gastropods^{31,32} and some extinct armoured heterostracan fishes³³). Although juveniles are more vulnerable than adults to most predators⁹, a heavier skeleton could limit the rate of body growth, increasing the time spent at smaller, more vulnerable sizes⁸. When mature and slow growing, skeletal reinforcement would no longer restrict potential growth. Where there is a refuge in size from predation⁹, trade-offs between rate of growth and extent of morphological defence may represent an important compromise.

Step(s) which limit the rate of skeleton production are unknown. Neither rates of ion transfer across epithelia³⁴ nor availability of dissolved calcium carbonate in surface seawater (<100 m; refs 35–37) are likely to limit the rate of skeletal growth. A probable limiting step is the rate at which crystals can grow from a biologically maintainable precipitating medium. If this is the case, during rapid growth crystal growth limitations would favour shell microstructures with many small crystals because of the increased surface for precipitation. Limited data reveal such a pattern in comparisons of seasonally rapid and slow growth increments in two bivalve species³⁸. Furthermore, both bivalved^{4,39} and shelled gastropod³⁹ molluscs exhibit an evolutionary loss of nacre, a shell microstructure composed of large crystal elements. Thus both ecological and evolutionary patterns in skeletal architecture may be explained by the skeleton-limitation hypothesis.

The above evidence indicates that a potentially important non-energetic 'cost' exists due to growth constraints imposed by carbonate skeletons, both for marine gastropods and possibly for other marine invertebrates. The prevailing attempt to explain morphological adaptations purely in terms of energetic costs and benefits may be too narrow a perspective.

I thank E. G. Boulding, M. LaBarbera, R. B. Lowell, R. E. Peter and A. N. Spencer for reading and criticizing early drafts of this manuscript. This research was funded by NSF grant OCE-77-25901-02 to R. T. Paine. Logistical support while writing was provided by NSERC operating grant A7245 to the author.

Received 30 December 1980; accepted 18 March 1981.

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Segment-specific organization of leg motoneurons is transformed in bithorax mutants of *Drosophila*

Steven H. Green

Division of Biology 216-76, California Institute of Technology, Pasadena, California 91125, USA

In *Drosophila*, genes controlling segmentation in the thorax and abdomen are clustered in one region of the genome known as the bithorax complex. Studies of the genetics of this complex suggest that loss of activity of a gene causes transformation of a particular segment to a more anterior one, mesothorax representing the ultimate transformation¹. This transformation is well described for the epidermis, but it is not clear whether other segmentally arranged tissues are also transformed. The segmental ganglia are fused in *Drosophila* into a single compact mass termed the thoracic ganglion but the segmental organization of the nervous system is still apparent. There are discrete regions of neuropil, termed neuromeres, corresponding to the three thoracic segments: prothorax, mesothorax and metathorax. A small terminal neuromere corresponds to the abdominal segments. Evidence is presented here that the leg motoneurons of each of the three thoracic segments are arranged in a segment-specific pattern in the thoracic ganglion. In mutant flies which have the metathoracic cuticle transformed to mesothoracic, the arrangement of the metathoracic leg motoneurons can be altered to resemble that of the mesothoracic leg motoneurons.

Horseradish peroxidase (HRP) filling of cells was used to study the innervation of the leg. This enzyme is applied to a cut made in the tarsus and is transported through those sensory axons that have been cut. It is also taken up by motoneurone terminals and transported in a retrograde fashion to fill the entire cell. The HRP-filled cells can be visualized by any one of several methods^{2,3}. Figure 1 is a composite drawing showing leg motoneurons in each neuromere. The arrangement of the leg motoneurons is different in the three neuromeres: in the prothoracic (anterior) neuromere, the cell bodies are in a region of the cortex anterior to the neuropil. The axons project back into the neuropil, into which they branch, collect into a loose bundle and course laterally and ventrally to leave the ganglion in the prothoracic leg nerve. In the mesothoracic (middle) neuromere, the cell bodies are in the cortex anterior and ventral to the neuropil. The axons project posteriorly to exit through the mesothoracic leg nerve. Branches are directed dorsally into the lateral neuropil, segregated from sensory neurone arborizations which are in the medial region. In the metathoracic (posterior) neuromere, the motoneurone cell bodies lie laterally and somewhat posteriorly to the neuropil. Their axons project anteriorly from the cell bodies, then loop back and run posteriorly to the nerve. All the axons loop back at the same point although the cell bodies are spread through a region 30–60 µm posterior to the loop. As in the mesothoracic neuromere, the arborizations of the motoneurons are laterally placed in the neuromere, not overlapping the medially placed arborizations of the sensory neurones. In each neuromere a single motoneuron sends a branch contralaterally. These cross in

commissures located posteriorly in the prothoracic and mesothoracic neuromeres but anteriorly in the metathoracic neuromere. In the mesothoracic neuromere this motoneurone has its cell body located anteriorly to the other motoneurone cell bodies. Thus, in terms of the positions of the leg motoneurone cell bodies and of the commissure, the metathoracic neuromere is the reverse of the anterior two neuromeres along the anterior-posterior axis.

Similar results were obtained by cobalt filling of axons at the cut end of the coxa, using a method described elsewhere⁴. Furthermore, observations of silver-stained ganglia by Power⁵ and repeated in this laboratory reveal large cells with an arrangement and nuclear morphology like that of the backfilled motoneurones. Therefore, the differences in the motoneurone pattern in the three neuromeres do not seem to result from uptake of HRP by different subpopulations of an identically arranged motoneurone population in each neuromere. The arrangement of the leg motoneurones as described above is the same in males and females of the *Drosophila melanogaster* wild-type strains Canton-S and Oregon-R, the mutant spineless-aristapedia and *Drosophila virilis*.

The bithorax complex mutations *abx* (ref. 6) and *bx* transform anterior metathorax to anterior mesothorax; *pbx* does the same to the posterior compartment. Flies of the genotypes *abx bx*³ *pbx/abx bx*³ *pbx* and *abx bx*³ *pbx/Df(3R)P2* (ref. 7) (derived from stocks provided by Dr E. Lewis) can have the cuticle of the metathoracic segment almost identical to that of the mesothoracic, although expression (degree of change) is variable and incomplete transformations are seen. In all cases studied, the

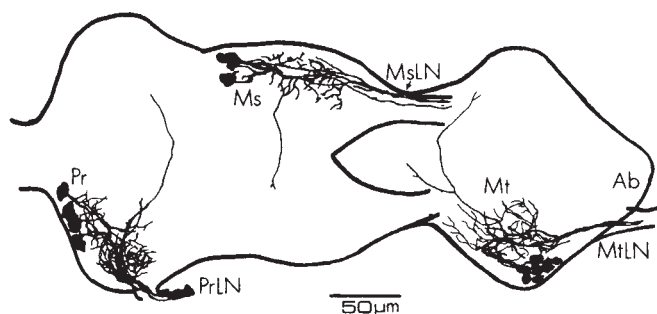


Fig. 1 A wild-type thoracic ganglion is shown in horizontal plane. This drawing is a composite of three separate backfills, traced from photomicrographs on to one thoracic ganglion outline. Only one side is shown for each neuromere. As many as nine motoneurones have been filled from a given leg but generally fewer are seen as in the pro- and mesothoracic examples shown here. In anterior to posterior order, the prothoracic (Pr), mesothoracic (Ms), metathoracic (Mt) and abdominal (Ab) neuromeres are labelled. Leg nerves: PrLN, prothoracic; MsLN, mesothoracic; MtLN, metathoracic. Sensory axons filled in these preparations were not included in the drawings. The motoneurones were filled by applying 20% HRP + 3% α -lysocitinin to a cut at the distal end of a leg. Backfill time was 10–14 h. The ventral thorax was removed and fixed with 1.25% glutaraldehyde + 1% formaldehyde in 0.1M phosphate buffer (pH 7.3) for 30 min. The ganglion was removed from the cuticle, fixed for an additional 45 min and washed three times for 30 min in phosphate buffer + 10% sucrose. The HRP was visualized by one of the following two methods: (1) The ganglion was washed two times for 5 min in Hanker-Yates reagent (1.5 mg ml⁻¹ in 0.05M Tris buffer, pH 7.6), and then incubated for 3–6 min in fresh reagent + 0.006% hydrogen peroxide. The reaction, which colours the HRP-filled neurones reddish-brown, was observed with a dissecting microscope to determine the appropriate end point. The ganglion was then washed three times for 5 min in Tris buffer, dehydrated in ethanol (1 min each 70% and 95%, three times for 1 min in 100%), cleared in methyl salicylate and mounted in immersion oil. (Method modified from ref. 2.) (2) The ganglion was kept for 15 min in a medium made up as follows: 0.25 ml of 3,3', 5,5'-tetramethylbenzidine solution (2 mg ml⁻¹ in 100% ethanol) was added to 10 ml of sodium ferricyanide solution (1 mg ml⁻¹ in 0.01M acetate buffer, pH 3.3), both solutions freshly made up. Hydrogen peroxide was then added to a final concentration of 0.006% and the ganglion kept in this for 4–6 min. This reaction colours the HRP-filled neurones blue. As above, the actual reaction time was determined by observing the reaction with a dissecting microscope. The ganglion was washed three times for 5 min with acetate buffer and further processing was as described above. (Method modified from ref. 3.)

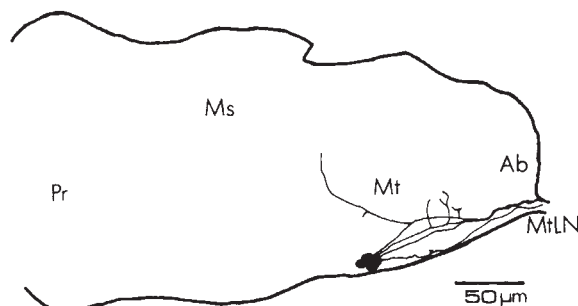


Fig. 2 A thoracic ganglion from an animal of genotype *abx bx*³ *pbx/abx bx*³ *pbx* is shown in horizontal plane. A metathoracic leg was backfilled and the HRP visualized as for method (1) in Fig. 1 legend. Labelling as in Fig. 1.

metathoracic leg itself was completely transformed into a mesothoracic leg, as determined by the pattern of leg bristles. Metathoracic legs of these flies were cut and HRP applied as for the wild-type flies. Of 27 successful backfills, four showed altered patterns of motoneurone arrangement that resembled mesothoracic motoneurones and nine showed intermediate phenotypes; unusually positioned motoneurones, suggestive of a mesothoracic pattern but resembling neither pattern entirely. The remaining 14 backfilled legs showed the wild-type arrangement of motoneurones. Of 41 successful metathoracic leg backfills in flies wild-type for bithorax, no deviations from the metathoracic pattern were ever observed. This is consistent with the hypothesis that the central nervous system (CNS) can be segmentally transformed by the mutations. As with the epidermis, expression is variable although the expression and penetrance (fraction of flies affected) of the mutations are far less in the CNS than in the cuticle.

Figure 2 exemplifies a metathoracic neuromere with a mesothoracic pattern of motoneurone arrangement. The motoneurone cell bodies lie anteriorly and ventrally in the cortex and the axons project posteriorly through the neuropil. Figure 2 also shows that the cell which projects contralaterally is anterior to the other cells, just as in the mesothoracic neuromere. Thus, a unique metathoracic neurone seems to be converted into its mesothoracic homologue. Nevertheless, the transformation to the mesothoracic is not complete. The contralaterally projecting fibre takes an anterior route as in the wild-type metathoracic neuromere.

Two examples of intermediate phenotypes are shown in Fig. 3: *a* shows a metathoracic neuromere containing motoneurones in intermediate positions; *b* shows a ganglion with metathoracic leg motoneurones that are positioned properly but have axons that project posteriorly into the nerve instead of looping anteriorly. Possibly, cell body position and direction of axon growth are under separate control by anterior-posterior information.

Previous neuroanatomical studies^{8,9} of bithorax using these mutants to study sensory projections from the ectopic cuticle raised the question of whether the mutations affect the CNS itself. Palka *et al.*⁸ found sufficient differences between bithorax and wild-type ganglia and sensory pathways to suggest that the CNS was altered by the mutations. They found additional evidence for this by considering the pattern of projection of sensory neurones from clones of mutant cuticle into presumably wild-type ganglia. Ghysen⁹, stressing the overall anatomical similarity of wild-type and bithorax ganglia and sensory pathways concluded that the CNS was not altered in the specific pathways studied.

I have approached the question directly by studying neurones with cell bodies in the CNS, using the recently constructed combination *abx bx*³ *pbx* to obtain more extreme transformations than were previously available. The observations reported here do not imply that the genes of the bithorax complex directly control the CNS segmental pattern. They may

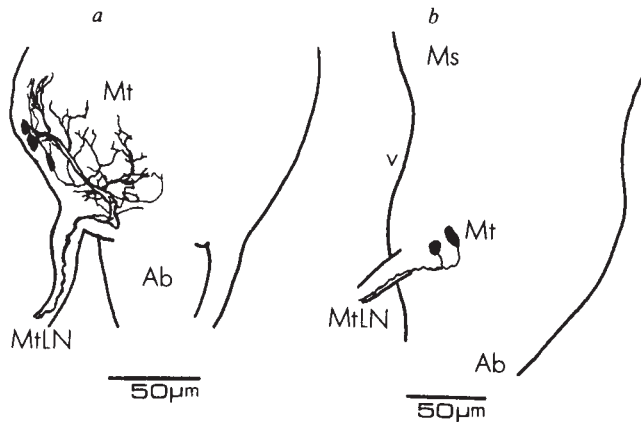


Fig. 3 Two 'intermediate' transformations. *a*, A thoracic ganglion from an animal of genotype *abx bx³ pbx/abx bx³ pbx* in horizontal plane. A metathoracic leg was backfilled and the HRP visualized using method (1) of Fig. 1 legend. *b*, A thoracic ganglion from an animal of the genotype *abx bx³ pbx/Df(3)P2* in sagittal plane, V marking the ventral surface. A metathoracic leg was backfilled and the HRP visualized using method (2) of Fig. 1 legend. Labelling as in Fig. 1.

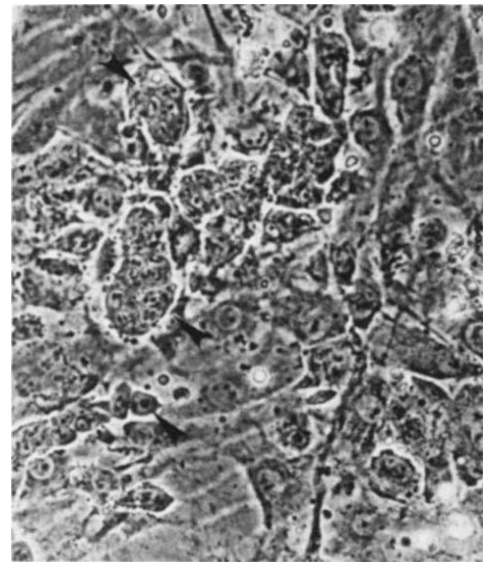


Fig. 1 Groups of pluripotent embryo cells (arrowed) growing in monolayer culture on a background of mitomycin C-inhibited STO cells. The isolation of a definite cell line from a blastocyst takes only ~3 weeks and the pluripotent cell colonies are visible within 5 days of passage. We have had 30% yield of lines from blastocysts in one experiment. Two of the lines have been rigorously cloned by single-cell isolation but most were only colony-picked—this makes no difference.

result from a direct effect of the genes in another tissue, which in turn affects the CNS by induction or by mechanical constraints on CNS growth. Experiments using mosaic flies should determine which tissue must be mutant for the pattern of organization of leg motoneurons to be transformed. This should help to elucidate the mechanisms underlying segmental differences in the CNS.

I thank Dr Ronald Konopka for advice throughout this work and A. Ferrus, E. Lewis, E. Meyerowitz and S. Shotwell for comments on the manuscript. This work was supported in part by a National Research Service Award (1 T32 GMO7737) from the National Institute of General Medical Sciences and a Spencer predoctoral fellowship, in addition to USPHS grants GM 22227 and AG 01844 to Ronald Konopka.

Received 20 January; accepted 9 April 1981.

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Establishment in culture of pluripotent cells from mouse embryos

M. J. Evans* & M. H. Kaufman†

Departments of Genetics* and Anatomy†, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK

Pluripotent cells are present in a mouse embryo until at least an early post-implantation stage, as shown by their ability to take part in the formation of chimaeric animals¹ and to form teratocarcinomas². Until now it has not been possible to establish progressively growing cultures of these cells *in vitro*, and cell lines have only been obtained after teratocarcinoma formation *in vivo*. We report here the establishment in tissue culture of pluripotent cell lines which have been isolated directly from *in vitro* cultures of mouse blastocysts. These cells are able to differentiate either *in vitro* or after inoculation into a mouse as a tumour *in vivo*. They have a normal karyotype.

Previous attempts to obtain cultures of pluripotent cells directly from a mouse embryo have been unsuccessful^{3,4}, although cells with a similar appearance have been reported to be present transiently^{5,6}. We considered that success might depend on three critical factors: (1) the exact stage at which pluripotent cells capable of growth in tissue culture exist in the embryo; (2) explantation of a sufficiently large number of these precursor cells from each embryo; and (3) tissue culture in conditions most conducive to multiplication rather than differentiation of these embryonic cells. These considerations have been discussed at greater length elsewhere⁷. An indication of the optimal stage of embryonic development might be gained by a comparison of the properties of embryonic cells at various stages with established cultures of embryonal carcinoma (EC) cells. Cell-surface antigen expression and the patterns of protein synthesis revealed by two-dimensional electrophoresis have suggested that neither the cells of the 6½-day ectoderm nor those of the 3½-day inner cell mass show homology with EC cells, but that epiblast cells of the early post-implantation embryo at 5½ days post coitum may do so⁸ (the day of finding coital plug is termed day ½). Cells from embryos of an early post-implantation stage seem to be the best candidates for direct progenitors of pluripotent cells in culture. As these embryos are difficult to isolate, and as the cell number in the isolated epiblast is small, we chose an alternative route to obtain embryo cells at this stage of development.

Mouse blastocysts may be induced to enter a state of diapause just before implantation. This delay in implantation depends on the maternal hormonal conditions, and may be induced experimentally by ovariectomy at an appropriate stage⁹. Embryos in implantational delay hatch from the zona but remain free-floating in the uterine lumen. A gradual increase in cell number occurs¹⁰, and the primary endoderm may be formed but no further development takes place until implantation occurs, under the control of hormonal stimuli.

129 SvE mice were caged in pairs and examined for mating plugs each morning. They were ovariectomized on the afternoon of day 2½ of pregnancy, injected subcutaneously with 1 mg Depo-Provera (Upjohn), and delayed blastocysts were recovered 4–6 days later. The blastocysts were cultured intact in

groups of about six embryos in small drops of tissue culture medium under paraffin oil on tissue culture plastic Petri dishes for 4 days. The blastocysts attached within 48 h and the trophoblast cells grew out and differentiated into giant trophoblast cells. The inner cell mass cells subsequently developed into large egg cylinder-like structures, with a group of small round cells surrounded by endodermal cells growing attached to the Petri dish. The egg cylinder-like structures were picked off the dish, dispersed by trypsin treatment and passaged on to gelatin-pretreated Petri dishes containing mitomycin C-inactivated STO fibroblasts. All culture was carried out in Dulbecco's modified minimal essential medium supplemented with 10% fetal calf serum and 10% newborn calf serum. The cultures were examined daily and passaged by trypsinization every 2–3 days. Actively proliferating colonies of cells closely resembling EC cells were apparent from an early stage. These colonies were picked out, passaged and mass cultures grown. The cell cultures had the appearance and general growth characteristics of feeder-dependent EC cells (Fig. 1).

The embryos used to initiate these cultures are from normal 129 SvE strain mice, that is, from the same strain of mice as many EC cell lines, in particular those grown in this laboratory. Therefore it was important to exclude any possibility of contamination of these cultures with EC cells from established cell lines. Cell cultures were established from different embryos in three separate experimental series, but the best indication of their separate identity came from their karyotype. Cultures were initiated from 6–12 embryos, thus it might be expected that both male and female cells should be present. None of the 129 embryonal carcinoma cell lines in this laboratory have a normal karyotype, and, in particular—in common with most available embryonal carcinoma cell lines—they do not contain a Y chromosome. These embryo-derived cells have a completely normal karyotype. An XY karyotype is shown in Fig. 2. Three additional cell lines have been analysed; two of these are normal 40XX and one is normal 40XY. We have termed these directly embryo-derived cells EK to distinguish them from EC cells. EK cells grow rapidly in culture and have been maintained for over 30 passages *in vitro*.

Cultures of EK cells were collected by trypsinization, and $\sim 10^6$ cells injected subcutaneously into the flank of syngeneic male mice. Tumours grew in all cases, and histological examination of these revealed that they were teratocarcinomas. When the EK cells were passaged without feeder cells they formed embryoid bodies which, when kept in suspension, became cystic.



Fig. 2 Karyotype of an embryo-derived pluripotent cell line, 40XY. Over 80% of the spreads of this clonal line possessed 40 chromosomes and had a clearly identifiable Y chromosome.

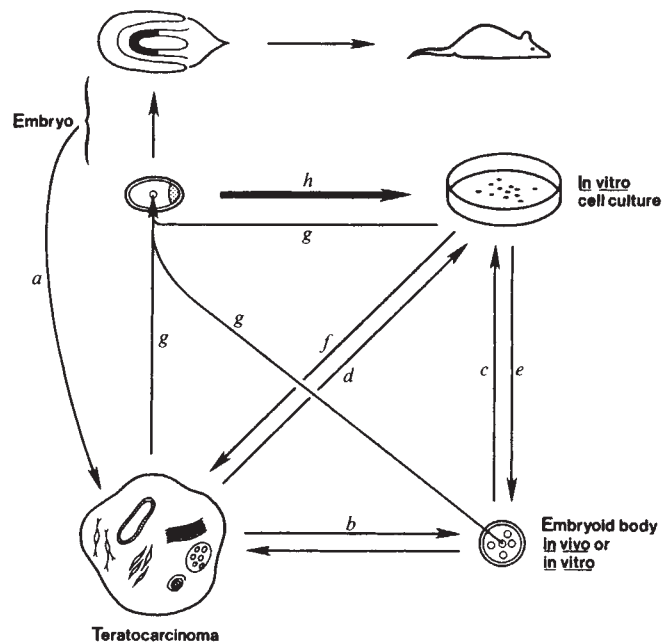


Fig. 3 Inter-relationships of cell lines, teratocarcinomas and embryoid bodies with normal mouse embryos. Arrows indicate routes of cell transfer: *a*, formation of teratocarcinoma by ectopic implantation of embryos; *b*, formation of embryoid bodies from teratocarcinoma and vice versa; *c*, derivation of cell culture from embryoid bodies; *d*, cell culture obtained directly from solid tumours; *e*, differentiation to embryoid bodies from culture; *f*, formation of solid tumours on reinjection of cells from culture; *g*, transfer of embryonal carcinoma cells either from cell culture or from the core of an embryoid body or from a solid tumour back to a blastocyst. All these procedures may result in chimaerism of the resulting mouse; *h*, the missing link supplied here.

Embryoid bodies allowed to attach to a Petri dish spread out and differentiated in the usual way into a complex of tissues. Preliminary observations indicate that, like early ectoderm cells of the mouse embryo and EC cells, EK cells carry the cell-surface antigens recognized by M1-22-25 (Forssman)^{8,11} and anti-I Ma (lacto-*N*-iso-octaosyl ceramide)^{12,13} and also that two dimensional gel electrophoretic separations of their proteins very closely resemble those of the EC cell line PSMB.

We have demonstrated here that it is possible to isolate pluripotent cells directly from early embryos and that they behave in a manner equivalent to EC cells isolated from teratocarcinomas. The network of inter-relationships between the mouse embryo and pluripotent cells derived from it has previously lacked only the direct link between the embryo and cells in culture for completion. We have now demonstrated this (Fig. 3).

Teratocarcinoma cells are now being widely used as a model for the study of developmental processes of early embryonic cell commitment and differentiation. Their use as a vehicle for the transfer into the mouse genome of mutant alleles, either selected in cell culture or inserted into the cells via transformation with specific DNA fragments, has been presented as an attractive proposition. In many of these studies the use of pluripotent cells directly isolated from the embryos under study should have great advantages. We have now shown that these EK cell lines are readily established from cultures of single blastocysts and so far have 15 lines of independent embryonic origin, some of which have been isolated from non-129, outbred mouse stocks. We are now studying the chimaeric mice formed from these cells.

We thank Mrs A. Burling for technical assistance and Dr E. P. Evans for advice regarding karyotype analysis. M.J.E. and M.H.K. were supported by the MRC; M.J.E. also received support from the Cancer Research Campaign.

Received 6 February; accepted 14 April 1981.

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Stage-specific embryonic antigen involves $\alpha 1 \rightarrow 3$ fucosylated type 2 blood group chains

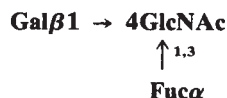
H. C. Gooi*, T. Feizi*, A. Kapadia*, B. B. Knowles†, D. Solter† & M. J. Evans‡

* Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, UK

† Wistar Institute of Anatomy, Philadelphia, Pennsylvania 19104, USA

‡ Department of Genetics, University of Cambridge, Cambridge CBQ 3EH, UK

There is much interest in developmentally regulated molecules which may have function in cell interactions and sorting during embryogenesis and differentiation. Numerous antisera have been raised which detect antigens that are expressed in early embryonic cells and become restricted during differentiation, being expressed in only a minority of adult cells (reviewed in refs 1–3). The precise antigenic determinants recognized by such antisera have not been defined. However, studies using a hybridoma antibody against mouse spleen cells⁴ and monoclonal autoantibodies of patients with cold agglutinin disease⁵ have shown that two defined carbohydrate antigen systems, the Forssman and the Ii antigens, have stage-specific expression in early mouse embryos. We now describe evidence that the stage-specific embryonic antigen SSEA-1 (ref. 6) involves the carbohydrate sequence



This determinant is formed by $\alpha 1 \rightarrow 3$ fucosylation of blood group I or i antigens which are branched or linear oligosaccharides, respectively^{7–9}, built of $\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}$ units and known as type 2 precursor chains¹⁰ of the major blood group antigens. Thus, we introduce the concept of simple glycosylation changes as a basis for stage-specific expression of embryonic antigens.

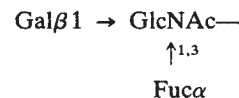
A relationship between the stage-specific embryonic antigen, SSEA-1, and blood group I antigen was suggested by: (1) some similarities in the distribution of these two antigens in early post-implantation embryos and teratocarcinomas^{5,11}, and (2) the reactivities of anti-I and -SSEA-1 reagents with a glycosphingolipid fraction of erythrocytes¹². Subsequent immunocytochemical studies (to be described elsewhere) have clearly shown that the two antigens are not identical. Furthermore, in binding assays using radioiodinated blood group I-active glycoproteins¹³ isolated from sheep gastric mucins and human meconium, the anti-SSEA-1 reagent gave a binding curve only with the latter glycoprotein (Fig. 1).

The specificity of anti-SSEA-1 reagent was further investigated by inhibition assays using blood group substances with known A,B,H,Lewis^a, Lewis^b, I and i activities, some of which

are shown in Fig. 1. These substances were clearly divisible into two groups: those with potent inhibitory activities, giving 50% inhibition at $0.3\text{--}6\ \mu\text{g ml}^{-1}$, and those with negligible or weak activities requiring concentrations $>100\ \mu\text{g ml}^{-1}$ for 50% inhibition. Almost all the substances with strong SSEA-1 activity lacked blood group ABH activities but expressed Le^a and/or Ii activities.

The two ovarian cyst glycoproteins with the strongest SSEA-1 activity, designated N-1 10% and F1, contained 8.5% and 1.6% fucose, respectively^{14,15}. In experiments to be described elsewhere, we showed that their reaction with anti-SSEA-1 was abolished by mild acid hydrolysis. Thus, it seemed likely that the SSEA-1 determinant involved fucose residue(s) and that the fucosyl linkage was other than those associated with A, B, H, Le^a or Le^b activities¹⁰. This agrees with previous evidence for the presence of unusual fucosyl glycopeptides²⁰ in undifferentiated cells of mouse embryos and teratocarcinomas.

Inhibition of binding assays were next performed with chemically synthesized^{21–23} and natural oligosaccharides^{14,24} containing type 1 ($\text{Gal}\beta 1 \rightarrow 3\text{GlcNAc}$) or type 2 ($\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}/\text{Glc}$) precursor chain¹⁰ sequences (Fig. 2). Whereas Ii-active synthetic tri- and pentasaccharides consisting of type 2 precursor chains were inactive as inhibitors of anti-SSEA-1, several fucose-containing oligosaccharides showed inhibitory activities. The most active inhibitor (ID_{50} 0.5 nmol) was the oligosaccharide designated N-1 R_L 0.71a, containing a fucosylated type 2 sequence:



This oligosaccharide was one of several isolated from glycoprotein N-1 after partial alkaline degradation¹⁴. The very poor inhibitory activity of 3-fucosyl lactose indicated that subterminal N-acetyl glucosamine is an important component of

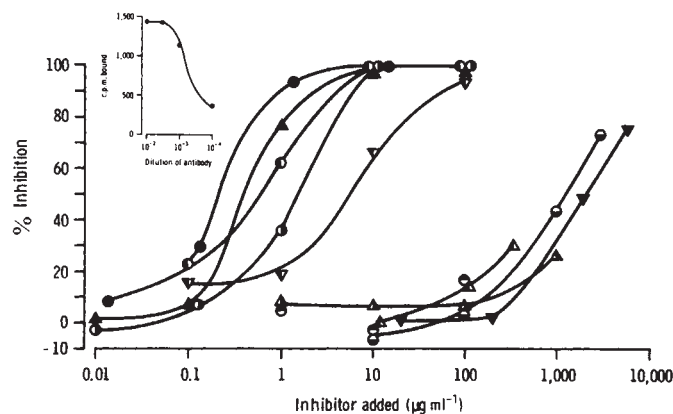
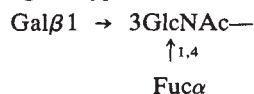


Fig. 1 Double antibody radioimmunoassay using anti-SSEA-1 and ¹²⁵I-labelled human meconium. The immunoassay procedure was a modification of that described previously¹³. The anti-SSEA-1 reagent^{6,11} was ascites fluid from a pristane-primed BALB/c mouse injected with antibody-producing hybrid cells. Normal mouse serum, 1:100 dilution, was used as carrier and undiluted rabbit anti-mouse immunoglobulin serum (Dako-immunoglobulins) as second antibody. The binding curve is shown in the inset. Inhibition assays were performed using the anti-SSEA-1 reagent at 1:3,000 dilution. As inhibitors blood group substances of human and animal origins were used. Symbols, blood group activities and designations (the latter in parentheses) of representative samples are given below. The following were human ovarian cyst glycoproteins: ●, Le^aI (N-1 10%)¹⁴; ▲, Ii (F1)¹⁵; ○, Ii (484)¹³; ▼, Le^a (445); △, HLe^b (JS)¹⁶; ⊙, A (438)¹³. Also tested were gastric mucosal glycoproteins from sheep, ▲, Ii (sheep 1 + 10)¹⁷ and from hog, ▼, AH (hog A + H)¹⁸, a glycoprotein-rich extract from human meconium of non-secretor type, ○, Le^aIi (Mec)¹³ and polyglycosyl ceramides isolated from human erythrocytes, ⊙, HII (ref. 19).

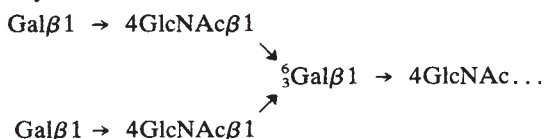
the SSEA-1 antigenic determinant. The Le^a-active oligosaccharides tested having the type 1-based terminal sequence



were almost 10 times less active than their type 2 isomers. Lacto-*N*-difucohexaose I (Le^b active), lacto-*N*-fucopentaose I (H active) and 3-fucosyl lactose were 200–1,000 times less active as inhibitors; 2-fucosyl lactose, lacto-*N*-tetraose and lacto-*N*-neotetraose were inactive.

Further investigation is required to determine whether the weak inhibitory activity observed with the Le^a-active oligosaccharide represents a cross-reaction or whether it is due to the presence of their type 2 isomers in the preparations tested. It is also of interest to determine whether the 3-fucosyl N-acetyl lactosamine structure is the entire antigenic determinant recognized by anti-SSEA-1 or whether, as with Ii antigens^{7-9,25}, additional internal structures are involved. A recent observation¹² that a mixture of erythrocyte glycosphingolipids (H4 fraction) with blood group H and I activities precipitates with anti-SSEA-1, indicated that the SSEA-1, H and I antigens might co-exist on the same macromolecules. Glycosphingolipids with 3-fucosyl-N-acetyl lactosamine sequence are known to accumulate in adenocarcinomas²⁶ but it has not been demonstrated on erythrocyte glycosphingolipids of the H1-4 series, although its presence in small amounts has not been completely ruled out (S. Hakomori, personal communication).

Previous studies^{3,27} have shown that the earliest embryo cells of the mouse and similarly, undifferentiated teratocarcinoma cells, express I antigen which is a branched oligosaccharide built of *N*-acetyl lactosamine units^{7,8} as follows:



Presumably, a proportion of these chains become glycosylated with fucose in $\alpha 1 \rightarrow 3$ linkage in the late eight-cell stage, when SSEA-1 activity is first found⁶. At the start of differentiation, i is expressed on visceral endoderm cells in addition to I (ref. 5) and SSEA-1 (refs 9, 11). The i antigen is a linear structure also built of *N*-acetyl lactosamine units⁷:



Subsequently, during embryogenesis and embryonal carcinoma cell differentiation, several types of differentiated epithelia are found which lack I, i and SSEA-1 activities. A reciprocal increase in blood group H activity has been demonstrated in some of these⁵. The relationship between the I, i, SSEA-1 and H antigens is schematized in Fig. 3. It is now well established that the I and i antigens are masked in the presence of the blood group H-active fucose in $\alpha 1 \rightarrow 2$ linkage to galactose^{9,28} and a similar conclusion can be reached from the present studies, with respect to masking of SSEA-1 antigen by such fucosylation. Alternatively, sialylation may occur at the terminal galactose; in $\alpha 2 \rightarrow 3$ linkage the sialic acid causes partial or complete masking of the I and i antigens^{7,8} and possibly the SSEA-1 antigenic determinants. We can extrapolate further and envisage that, depending on the relative activities of the various glycosyl transferases (and perhaps glycosidases) in individual differentiated tissues, the proportions of these several antigenic determinants will vary. For example, variation in fucosylation might explain the presence of SSEA-1 in some blastomeres but not in others^{1-3,6}.

Thus, simple glycosylation steps involving changes in proportions of linear and branched structures and their fucosylated or sialylated derivatives provide a mechanism for stage-specific appearance and disappearance of antigens. It will be of interest to investigate which of the other various embryonic antigens¹⁻³ that disappear on differentiation reflect subtle glycosylation changes.

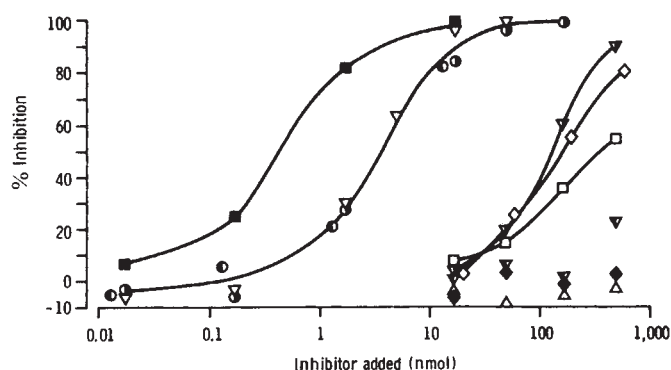
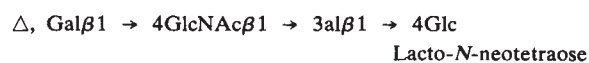
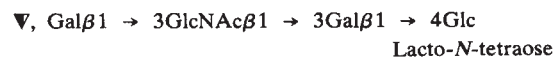
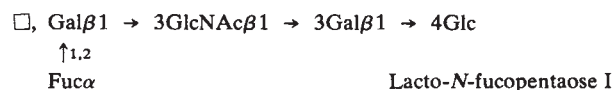
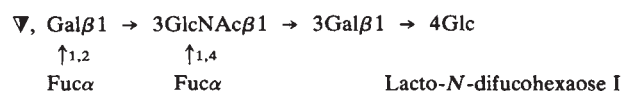
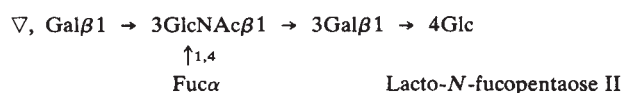
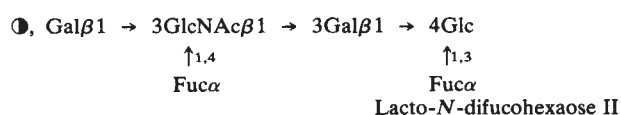
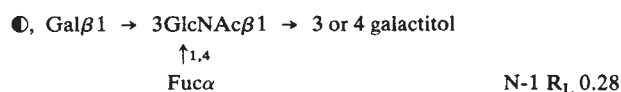
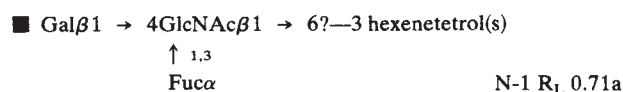
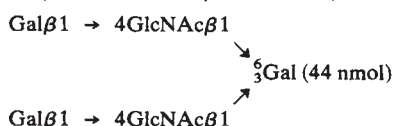
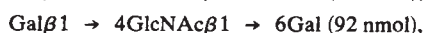
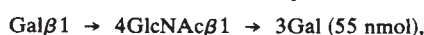


Fig. 2 Inhibition of binding of anti-SSEA-1 to 125 I-labelled meconium by oligosaccharides. Symbols and structures for the oligosaccharides in order of their inhibitory activities are as follows:



The following synthetic oligosaccharides²¹⁻²³ were inactive at the highest levels tested shown in parentheses:



Antigen activity				
I	i	SSEA	H	Other
	+	-	+	-
	-	+	+	-
	-	-	-	+
	-	-	-	+
	-	-	-	?
	-	-	-	?

Fig. 3 Schematic presentation of the proposed structure for the SSEA-1 antigenic determinant and its relationship to blood group I, i or H active oligosaccharides and to sialylated structures of undetermined antigenic specificity. Symbols: ○, $\beta 1 \rightarrow 4$ linked galactose; ●, $\beta 1 \rightarrow 3$ linked *N*-acetyl glucosamine; ⊙, $\beta 1 \rightarrow 6$ linked *N*-acetyl glucosamine; △, $\alpha 1 \rightarrow 3$ linked fucose; ▴, $\alpha 1 \rightarrow 2$ linked fucose; □, $\alpha 2 \rightarrow 3$ linked sialic acid; ± implies optional substitution with $\alpha 1 \rightarrow 3$ linked fucose, such that in the absence of other peripheral monosaccharides, the I, i or the SSEA-1 antigenic determinants are expressed. It is not known whether the $\alpha 1 \rightarrow 2$ fucosylation or the sialylation steps precede or follow the $\alpha 1 \rightarrow 3$ fucosylation of these precursor chains.

The biological significance of the orderly changes of these and the major blood group antigens ABH (ref. 29) during development and differentiation is unknown. If these various carbohydrate sequences function as recognition structures involved in cell interactions and tissue organization, it can be anticipated that there will be a progressively increasing list of complementary molecules (endogenous lectins) specifically reactive with them. From the present studies it can be predicted that a 3-fucosyl *N*-acetyl lactosamine-mediated recognition system, analogous to the previously described lectin-like compound on hepatocytes³⁰, is operating at the embryonic cell surface, and it is tempting to speculate that such a lectin may be involved in the process of compaction. This occurs at the eight-cell stage of the mouse embryo when blastomeres become tightly associated with each other and undergo substantial changes in their organization during differentiation³¹. Thus, there is now a need to investigate the significance of different carbohydrate structures and lectins^{32,33} on mammalian embryonic cells. They may be involved in sugar-protein interactions which are crucial and specific for phenomena as varied as receptor-mediated uptake of circulating glycoproteins^{30,34,35} and the homing of enzymes to appropriate intracellular compartments^{34,36}.

We thank Drs E. A. Kabat, W. M. Watkins, C. Augé, S. David, A. Veyrières and J. Kościelak for providing oligosaccharides and blood group substances, Mrs Jean Picard for serological assessment of meconium extracts, Dr Peter W. Andrews for helpful discussions, and Mrs M. Moriarty for secretarial assistance. H.C.G. is a fellow of the Arthritis and Rheumatism Council, UK, and A.K. and M.J.E. are supported by the Cancer Research Campaign, UK. This study was supported in part by the US grants TSH NIH CA 18470, CA-10815, HD-12847 and NSF grant PCM-7816177.

Note added in proof: Further studies to be described elsewhere (E. F. Hounsell, H. C. Gooi and T. Feizi, in preparation) have shown that the inhibition of anti-SSEA-1 by the type 1 oligosaccharide, lacto-*N*-fucopentaose II, is due to the presence of the type 2 isomer, lacto-*N*-fucopentaose III, in this preparation.

Received 19 January; accepted 30 March 1981.

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Erythrocyte membrane sidedness in lectin control of the Ca^{2+} -A23187-mediated diskocyte $\rightleftharpoons$ echinocyte conversion

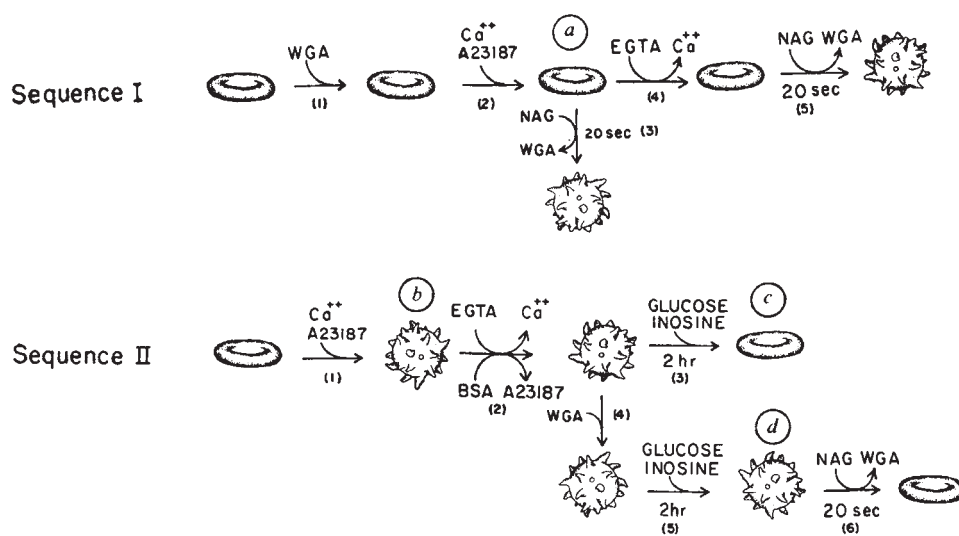
Richard A. Anderson & Rex E. Lovrien

Biochemistry Department, Gortner Laboratory, University of Minnesota, St Paul, Minnesota 55108, USA

Increasing the cytoplasmic calcium concentration of human erythrocytes with ionophore A23187 drives the transformation from diskocyte to echinocyte morphology¹. This transformation is closely linked to the intracellular ATP level, which drops when calcium is introduced across the membrane^{2,3}. The echinocyte morphology reverts to the diskocyte after restoration of normal ATP and calcium levels^{4,5}. ATP control of cell morphology is thought to depend on the action of a protein kinase and phosphatase which reversibly modify the membrane cytoskeleton⁶⁻⁹. The cytoskeleton in turn is in contact with the outside membrane surface via transmembrane proteins¹⁰⁻¹¹. We show here that binding of the lectin, wheat-germ agglutinin (WGA), to the erythrocyte membrane surface blocks the morphological conversions diskocyte $\rightleftharpoons$ echinocyte in both directions. WGA seems to work via a mechanism involving the transmembrane glycoprotein, glycophorin^{13,14}.

Wheat-germ agglutinin was used as a convenient tool to control in a reversible way the morphological conversion initiated by Ca^{2+} -A23187 on the cytoplasmic side. The internal echinocytic reactions initiated by Ca^{2+} -A23187 are not blocked by WGA binding to the membrane; instead they go to completion and are 'stored' until finally expressed, as a morphological change, when WGA is displaced from the membrane.

Fig. 1 Control of human erythrocyte morphology by wheat germ agglutinin (WGA) on the cell's outside surface in relationship to internal calcium loading and cell metabolism. Final erythrocyte concentration for sequence I is 1×10^7 cells ml^{-1} . Buffer (mM): 10 KCl, 130 NaCl, 2 MgCl_2 , 15 Tris, pH 7.4, 25 °C. Erythrocytes were drawn and washed as described before¹⁴. In sequence I the concentration of WGA is $2 \mu\text{g ml}^{-1}$; this results in 18×10^5 molecules bound per cell. In reaction (2), 0.2 mM Ca^{2+} and 5 μM A23187 were added, giving the final cytoplasmic calcium concentration at equilibrium of 0.4 mM. Addition of ionophore without added calcium caused no morphological change. Calcium influx into the cell was 50% complete after 30 s, 80% complete after 60 s and 100% complete after 100 s. The influx was followed using $^{45}\text{Ca}^{2+}$ (ref. 15). The binding of WGA to the cell did not change the flux of calcium across the membrane or the final cytoplasmic calcium concentration. Calcium was then withdrawn from the cell using 2 mM EGTA, leaving $< 10 \mu\text{M}$, 150 s after addition of EGTA. Lectin was displaced from the membrane by addition of 30 mM NAG. The unbinding was followed with ^{125}I -WGA¹⁴, and was complete ($< 2 \times 10^5$ molecules per cell) within 30 s of addition of NAG. In sequence II, diskocytes are converted to echinocytes using the above conditions. In reaction (2) calcium was removed with 2 mM EGTA and ionophore A23187 with a 0.3% bovine serum albumin wash. The red cells were $> 90\%$ echinocytes at this point, but upon re-energizing with 5 mM glucose and 2 mM inosine [reaction (3)], 95% of the cells reverted to diskocytes within 2 h. In reactions 4–6, the cell concentration was lowered to 2×10^6 cells per ml to avoid lectin-induced agglutination during the 2-h glucose-inosine incubation. The WGA concentration was $1 \mu\text{g ml}^{-1}$, with 20×10^5 molecules bound per cell.



The sequences of morphological changes are illustrated in Fig. 1. Figure 2 shows scanning electron microscope photographs of red cells in the critical stages of the reactions.

For the experiments of sequence I (Fig. 1), WGA was added to diskocytes such that the amount bound, ν_{WGA} , was 1.8×10^6 molecules of WGA per cell. This amount did not produce any change in cell morphology, as was expected¹⁴. When the cytoplasmic calcium concentration was increased, using 0.2 mM Ca^{2+} and 5 μM A23187, the cells with this level of bound lectin completely retained their normal diskocyte morphology. However, erythrocytes treated identically, but without lectin, lapsed to the echinocyte morphology within a few minutes. Figure 2a and b contrasts lectin-protected and unprotected cells.

The lectin does not control morphological conversion by blocking transport of calcium into the cell by ionophore A23187 because when the Ca^{2+} flux into the cell was quantified using $^{45}\text{Ca}^{2+}$ (ref. 15), both the rate of Ca^{2+} influx and the final cytoplasmic concentration of calcium were found to be identical for cells with bound lectin, and control cells.

WGA is rapidly displaced from protected cells by *N*-acetylglucosamine (NAG). Reaction (3) of sequence I shows that within 30 s of addition of NAG to lectin-protected Ca^{2+} -loaded diskocytes, the lectin was displaced and the diskocyte $\rightarrow$ echinocyte conversion completed. The velocity of this reaction was greater by an order of magnitude than that of the conventional Ca^{2+} -A23187-driven conversion (see Fig. 3), indicating that the erythrocytes were already calcium loaded when NAG was added.

Reaction (2) of sequence I shows that WGA completely blocks the echinocyte effects of Ca^{2+} -A23187. Reactions (4) and (5) show that the lectin does not block internal reactions initiated by Ca^{2+} . In reaction (4), EGTA was used to remove cytoplasmic Ca^{2+} . Using $^{45}\text{Ca}^{2+}$, removal of Ca^{2+} was shown to be complete (within experimental error of the measurement of cytoplasmic Ca^{2+} which is $\pm 10 \mu\text{M}$) within 150 s of addition of EGTA. The products of reaction (4) are diskocytes which have had Ca^{2+} -A23187 influxed, then completely removed, while fully protected by WGA. However, when WGA is displaced by NAG, as shown in reaction (5), the Ca^{2+} -free diskocytes

immediately change to echinocytes. Thus the internal reactions initiated by Ca^{2+} -A23187 are not erased by removal of Ca^{2+} , but their final expression, formation of echinocytes, is permitted only when the external lectin is removed.

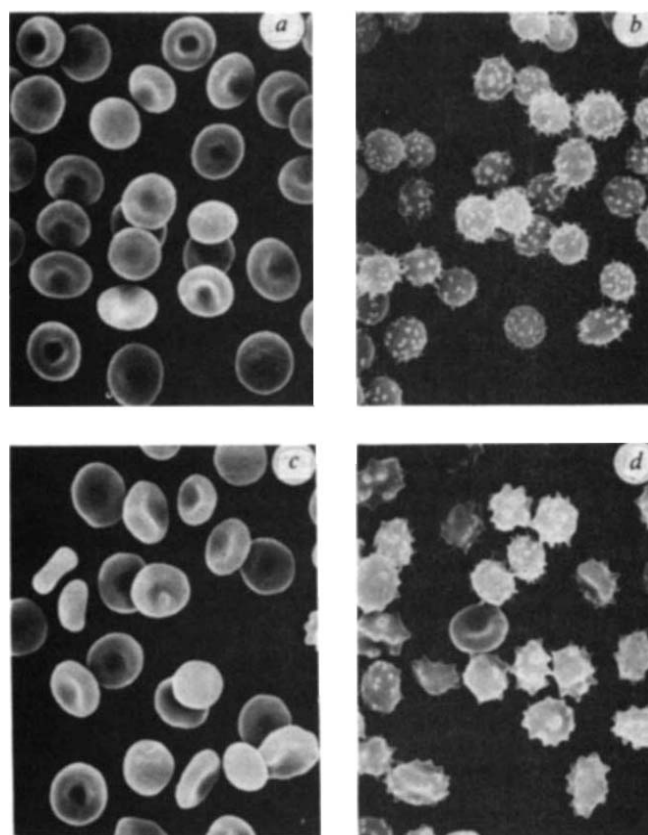


Fig. 2 Scanning electron micrographs of erythrocytes from stages indicated by the letters a, b, c and d in Fig. 1. Scale bar, 5 μm .

The effects of calcium on the cell morphology are reversible if the initial calcium concentration is <0.5 mM and the incubation time with calcium <15 min. In these conditions cytoskeletal cross-linking^{16,17} by endogenous transglutaminase or disulphide coupling⁵ does not occur to a significant extent. This is supported by the fact that the echinocyte morphology is reversed on restoration of normal calcium and ATP levels. Calcium also activates an endogenous phospholipase C which results in the hydrolysis of phosphatidylinositol and the accumulation of 1,2-diacylglycerol^{18,19}. In our conditions $<0.4\%$ ($<10^6$ molecules per cell) of the phospholipids would be hydrolysed¹⁹. It is unlikely that this small amount of 1,2-diacylglycerol would effect a morphological conversion²⁴.

The forward and reverse rates of the diskocyte $\rightleftharpoons$ echinocyte conversion in the absence of WGA and the profound decrease in these rates effected by the lectin are shown in Figs 3 and 4. The ordinate value in these figures, r , is the fraction of cells which are echinocytes; at $r = 0$, all cells are diskocytes.

Reaction (1) of sequence II represents a conventional diskocyte $\rightarrow$ echinocyte conversion by Ca^{2+} -A23187. When this conversion is 100% complete, the Ca^{2+} is removed by EGTA and the ionophore A23187 by serum albumin. If glucose and inosine are then added to allow regeneration of ATP, these calcium-unloaded echinocytes revert slowly to the diskocyte form. At least 95% diskocytes ($r = 0.05$) are obtained after 2 h.

In reaction (4) WGA is used to control the Ca^{2+} -A23187-produced echinocytes. If lectin is added to calcium-unloaded echinocytes followed by incubation in glucose for 2 h (the time necessary for completion of the conventional echinocyte $\rightarrow$ diskocyte conversion), the cell morphology does not change from the echinocyte. If the lectin is then displaced with NAG, the echinocyte $\rightarrow$ diskocyte conversion proceeds to completion within seconds. It is clear that the reactions needed to erase the impact of Ca^{2+} -A23187 on the membrane take place by the end of the 2-h incubation with metabolite. Thus displacement of WGA from echinocytes, which have been emptied of Ca^{2+} -A23187 and re-energized, causes a return to the diskocyte that is much more rapid than the conventional reversal in reaction (3).

The diskocyte $\rightleftharpoons$ echinocyte conversion is the result of a number of molecular events within the membrane. Increased cytoplasmic calcium activates internal reactions, which drive the change in cell shape. The data presented in Figs 3 and 4 indicate that the internal reactions for the diskocyte $\rightleftharpoons$ echinocyte morphological conversions are not blocked by lectin binding to the outside of the membrane. Instead lectin binding to one membrane component, which is crucial in the sequence of molecular events, blocks this component's participation in the sequence or pathway leading to morphological conversion, thereby completely blocking the conversion.

The receptor for WGA on the human erythrocyte is the major sialoglycoprotein, glyophorin^{13,14}. WGA binds to the sialic acid groups of glyophorin; when these groups are removed, lectin binding and control of cell morphology are eliminated¹⁴. Glyophorin is a transmembrane protein and is thought to be associated with band III within the membrane¹¹. It can thus interact with the cytoskeleton via the linkage band III-ankyrin-spectrin^{10,12}. Accordingly, WGA may influence the cytoskeleton and cell morphology, via the transmembrane proteins, in the following way:

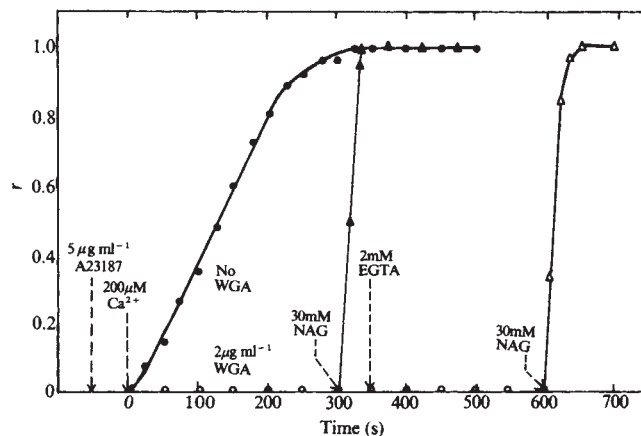


Fig. 3 Time course of the diskocyte $\rightarrow$ echinocyte conversions from sequence I, Fig. 1. r , Fraction echinocytes. Points lying on the abscissa ($r = 0$) represent WGA-protected diskocytes.

The existence of a cross-linking mechanism whereby the lectin randomly cross-links glyophorins to form an exocytoskeleton seems unlikely as: (1) freeze-fracture electron microscopy has shown that lectins do not cause clustering of receptors on intact cells^{20,21}, (2) the glycoprotein receptors are uniformly distributed in the membrane, ~ 200 Å apart²², and (3) the carbohydrate binding sites on WGA are 40 Å apart²⁵ and thus WGA could not span the distance separating the glyophorins. It is more likely that each WGA binds specifically to one glyophorin molecule, or, perhaps, to a dimer. Support for this view comes from work with peanut lectin, which can control change of shape of the desialated erythrocyte. Peanut lectin binds to desialoglycophorin with high specificity²³ and, at a concentration of 3×10^5 molecules bound per cell, completely blocks the diskocyte $\rightarrow$ echinocyte conversion. As there are only about 6×10^5 monomers of glyophorin per cell¹⁴, it is very

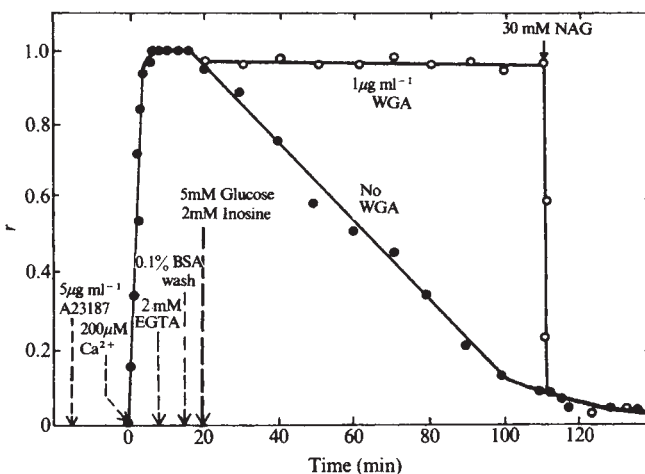
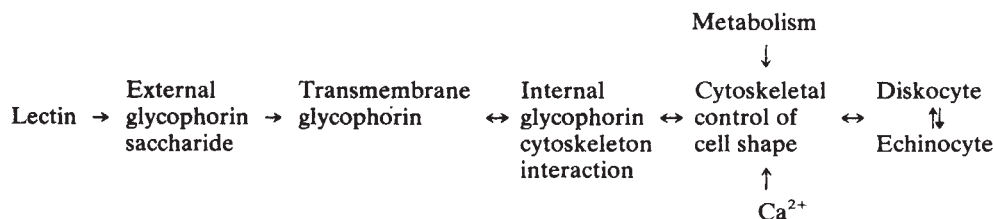


Fig. 4 Time course of the echinocyte $\rightarrow$ diskocyte conversions from sequence II, Fig. 1.



unlikely that random cross-linking takes place with such a small amount of lectin bound. A more reasonable proposition is that the lectins exert their control of morphology by intramolecular binding to a glycoprotein dimer. This is supported by the fact that the molecular ratio of peanut lectin: glycoprotein is 1:2 when morphology is completely blocked (R.A.A. and R.E.L. unpublished results).

The transmembrane protein glycoprotein is a crucial component in cytoplasmic Ca^{2+} -induced morphological change, and lectins may exert their control of morphology by specifically binding to glycoprotein. The 'sidedness' or asymmetric nature of glycoprotein enables carefully aimed use of lectins, specific for glycoprotein's external carbohydrate, to block the expression of internal biochemical reactions. The indirect action of Ca^{2+} on the cell cytoskeleton is paramount in this morphology conversion. Thus it is likely that glycoprotein-cytoskeletal contact is also important in this morphological conversion.

This work was supported by the University of Minnesota Graduate School.

Received 4 November 1980; accepted 12 March 1981.

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Cell extracts from the amoeboid stage of the cellular slime mould *Dictyostelium discoideum* contain protein factors that gel actin filaments *in vitro*⁹. We have recently purified to homogeneity one of these gelation factors that has a molecular weight of 120,000 (120K)¹⁰. It is a soluble, trypsin-sensitive and carbohydrate-free protein that increases the viscosity and sedimentation rate of F actin, inhibits the actin-stimulated Mg^{2+} -ATPase activity of myosin and nucleates actin filament assembly *in vitro*¹⁰. The interaction of this actin-binding protein with F actin is not inhibited by millimolar ATP or micromolar calcium. Electron microscopy demonstrates that this protein promotes both side-to-side and end-to-side interactions between actin filaments *in vitro*^{8,10}.

To ascertain the function of this protein *in vivo* we have used the indirect immunofluorescence technique to determine its location in spreading and migrating cells. Antisera were raised against purified native 120K protein in rabbits and characterized as monospecific by double diffusion and immunoelectrophoresis in agar¹¹ (Fig. 1). *Dictyostelium* amoebae were cultured in suspension in HLS¹⁰, washed in 20 mM KCl, 1 mM CaCl_2 , 2 mM

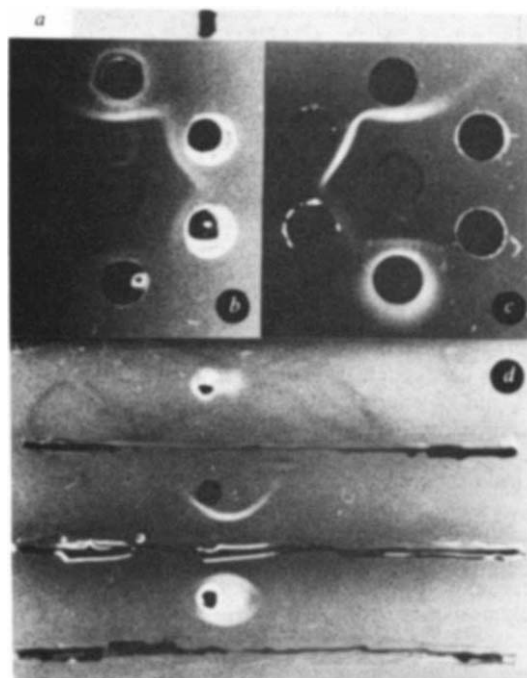


Fig. 1 The 120K protein was purified from *Dictyostelium* amoebae as described elsewhere¹⁰. **a** Shows SDS-polyacrylamide gel electrophoresis of the purified antigen. Rabbits were immunized with native 120K protein by subcutaneous injection of antigen in complete Freund's adjuvant and boosted once with antigen in incomplete Freund's adjuvant 4 weeks after initial immunization. Antiserum was characterized using double immunodiffusion in 1% agarose¹¹. **b** Shows result with antiserum in the centre well with a clockwise progressive twofold dilution of native 120K protein in the outer wells. **c** Shows the result with antiserum in the centre with outer wells filled with the following, clockwise from the top: a complex of 95,000 MW protein-120K protein-actin¹⁰; 95,000 MW protein alone; 95,000 MW protein plus actin; whole cell extract; 1/5th dilution native 120K protein; native 120K protein. In both cases single precipitin arcs result showing monospecificity and fuse to demonstrate immunological identity. No reaction was found in samples containing actin or 95,000 MW protein, the other major actin-binding protein found in *Dictyostelium*^{9,10}. **d**, Immunoelectrophoresis was performed as described previously in 1% agarose¹¹. Wells from top to bottom contain cell extract, native 120K protein and cell extract. Troughs from top to bottom contain pre-immune serum, antiserum to 120K protein and antiserum to 120K protein. A single precipitin line with the same electrophoretic mobility is formed against 120K protein and the cell extract.

A new protein that gels F actin in the cell cortex of *Dictyostelium discoideum*

John Condeelis & Jeffrey Salisbury

Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York 10461, USA

Keigi Fujiwara

Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02138, USA

It is thought that the dense actin filament networks found in the cortex of many cell types¹⁻⁴ might account for the gelled consistency of the cortex⁵ and the exclusion of other organelles from this region of the cytoplasm⁶⁻⁸. We now demonstrate that a new actin-binding protein from *Dictyostelium* that gels actin *in vitro* is concentrated in the cell cortex, suggesting that this protein, in association with actin, accounts for the gel-like properties of this region of the cell.

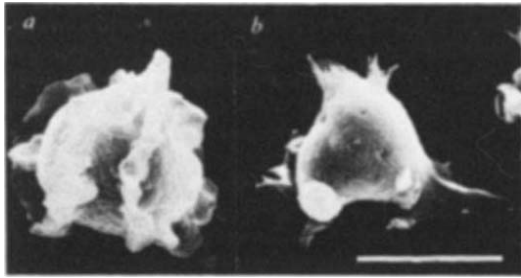


Fig. 2 Scanning electron micrographs of spreading (a) and migrating (b) amoebae on poly-L-lysine. Cells were prepared for microscopy by fixation in 2% glutaraldehyde in 40 mM sodium phosphate pH 7.0 for 10 min, dehydrated in ethanol and critical point dried against CO₂. Scale bar, 10 μ M.

MgSO₄ and 40 mM sodium phosphate pH 6.4 and allowed to spread and migrate on poly-L-lysine-coated coverslips. In these conditions, when the cells first make contact with the adhesive surface, they become flattened against the substrate on the bottom and proceed to throw ruffles from the surface back over the cytoplasm, which remains rounded (Fig. 2a). The cells then progressively flatten and begin to migrate by sending out large flat ruffles or pharopods (Fig. 2a). In randomly migrating cells such as those shown here the pharopods are usually extended from all sides of the cell with only one or two gaining dominance at the leading edge during net locomotion (Fig. 2b). Migrating cells usually remain slightly rounded in the cytoplasm region which contains the nucleus and other organelles, even when exhibiting rapid migration. At no stage during spreading or locomotion are stress fibres or large microfilament bundles observed within these cells in either the light or electron microscope, respectively⁸.

Immunofluorescence for 120K protein in cells undergoing various stages of spreading and migration on poly-L-lysine is shown in Fig. 3. All the cells have bound antiserum and exhibit at least one of two types of fluorescence: weak diffuse fluorescence which seems to be distributed throughout the cytoplasm exclusive of the nucleus, and strong peripheral fluorescence which is concentrated in the cell cortex, ruffles and pharopods. In spreading cells (Fig. 3c), ruffles at both the cell margin and those thrown back over the cytoplasm stain for the presence of 120K protein. In migrating cells (Fig. 3d), 120K protein is concentrated primarily in pharopods that extend randomly from the cell margin. In both cases a thin rim of fluorescence is usually seen around most of the periphery of the cell.

To determine whether 120K protein was present in mammalian cells, human (HeLa) and rat kangaroo (Ptk-2) tissue culture cells were fixed, permeabilized and stained with antiserum against 120K protein as described above. None of these cells demonstrated immunofluorescence for the protein.

The concentration of 120K actin-binding protein in structures associated with the cell periphery is analogous to the immunofluorescence localization in the cell cortex of macrophage actin-binding protein¹² and filamin¹³ in macrophages and cultured fibroblasts, respectively. The localization of 120K protein in the cell cortex, ruffles and pharopods of *Dictyostelium* is quite similar to that of macrophage actin-binding protein in lung macrophages¹² but less similar to the location of filamin in cultured fibroblasts, where this protein is also found in stress fibres. This latter difference might be due more to the absence of actin-containing stress fibres from *Dictyostelium* amoebae and macrophages than to dissimilarities in function between 120K protein and filamin.

The properties of purified 120K protein *in vitro* and its concentration in the cell cortex *in situ* suggest possible functions for this protein *in vivo*. The location of 120K protein in the cell corresponds well with that of actin determined by immunofluorescence¹⁴ and electron microscopy⁸. This protein

cross-links and gels filamentous actin and hence may be required with actin for the formation of the cortical gel long known to be present in amoeboid cells⁵. The presence of weak fluorescence for 120K protein throughout the cytoplasm of *Dictyostelium* amoebae suggests that small amounts of the protein in association with actin may also account for the presence of the diffuse filamentous network seen throughout the cytoplasm⁸. The concentration of 120K protein in ruffles and pharopods indicates that cytoplasm within these structures has an increased potential for the formation of a rigid actin gel but does not prove that the cytoplasm in this region is gelled continuously, because gels formed from 120K protein and actin *in vitro* are readily solated by micromolar Ca²⁺ in the presence of 95K protein^{6,9,10}.

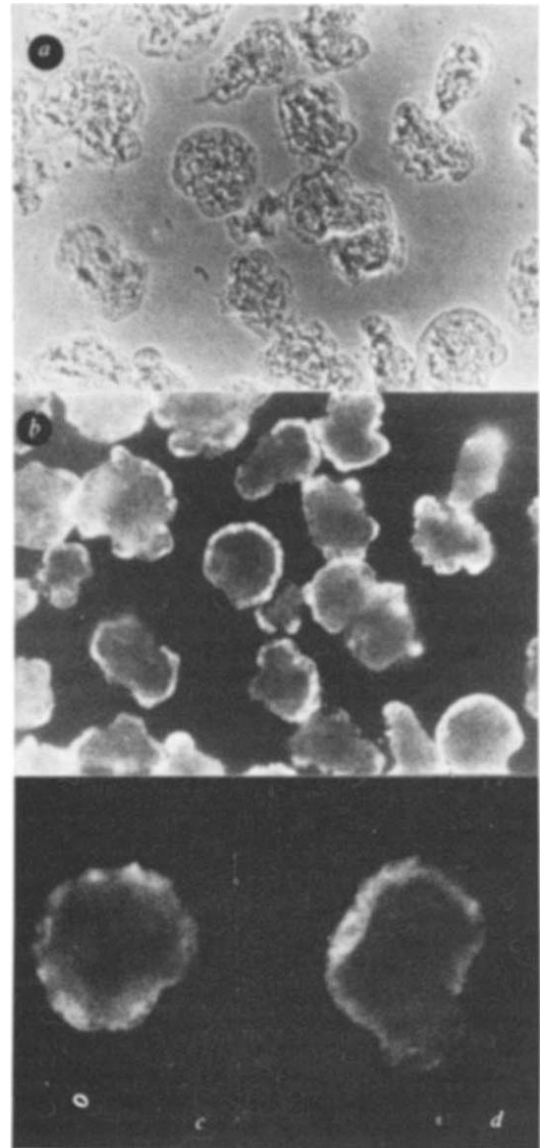


Fig. 3 Light microscopy of amoebae on poly-L-lysine. Cells were fixed in 3.7% formaldehyde in 0.85% NaCl, 10 mM sodium phosphate pH 7.2 for 10 min, permeabilized in -20°C acetone and stained with rabbit antiserum against 120K protein followed by goat anti-rabbit IgG conjugated to rhodamine (Cappel). Controls were as follows: pre-immune serum in place of antiserum against 120K protein, deletion of immune serum before staining with goat anti-rabbit IgG, cells fixed but not permeabilized with acetone, use of antiserum preabsorbed with native 120K protein. Staining was not detected in any of these controls. a, Phase and b, fluorescence of cells permitted to spread and migrate on poly-L-lysine; c, spreading cell, and d, migrating cell at higher magnification. a and b, $\times 1,200$; c and d, $\times 2,500$.

This protein also nucleates assembly of actin filaments, thereby promoting end-to-side filament attachments resulting in branched filaments which are frequently observed in the cortex of *Dictyostelium in situ*^{4,8}. Concentration in the cell cortex of actin nucleation activity associated with 120K actin-binding protein would have substantial significance in the generation of new actin filaments in this region of the cell during sol-gel transformations and pseudopod extension, and might result in the production of large numbers of free actin filament ends which could be used to advantage by the cell to establish membrane-filament attachments⁶.

We thank William Jordan Jr for technical assistance. This work was supported by grants from the USPHS to J. C. (GM 25290) and K. F. (GM 25637). J. C. is a Rita Allen Foundation Scholar. J. S. is an NIH Postdoctoral Fellow.

Received 19 January; accepted 31 March 1981.

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T cell-dependent IgA anti-polysaccharide response *in vitro*

Park E. Trefts, Denis A. Rivier & Martin F. Kagnoff

Department of Medicine, University of California at San Diego, La Jolla, California 92093, USA

The induction in mice of humoral antibody to purified polysaccharides, such as dextran B1355, dextran B512 and other bacterial capsular materials, is generally regarded as being thymus independent¹⁻⁵, that is, not requiring conventional carrier-specific T-cell help⁶. Such responses are largely immunoglobulin M (IgM), often transient although occasionally cyclic, and there is little evidence of immunological memory⁷⁻¹¹. The selective formation of an IgG response to polysaccharides or haptenated polysaccharides (for example, dextran B512, dinitrophenol (DNP)-Ficoll) has also been characterized as thymus independent^{12,13}. Recent reports have indicated that the murine IgG response to such antigens is restricted largely to the IgG₃ subclass^{14,15} although lesser amounts of other IgG subclasses have been reported after stimulation with thymus-independent antigens such as DNP-Ficoll and DNP-Levan^{16,17}. In the latter study, T cells were shown to influence certain IgG subclass responses to haptens on a thymus-independent carrier¹⁷. Here we report that BALB/c mice produce a substantial IgA anti- $\alpha(1,3)$ dextran B1355 response *in vitro* that is highly T-cell dependent and age-dependent. This finding represents a significant departure from previous observations and may have particular relevance for mucosal immunity. It also suggests the need for a re-evaluation of the induction and regulation of anti-polysaccharide antibody responses.

To study the induction and regulation of the Igh-1^a allotype-linked anti- $\alpha(1,3)$ antibody response to the purified polysaccharide dextran B1355 (43% $\alpha(1,3)$ and 57% $\alpha(1,6)$ glucan linkages) we used Mishell-Dutton *in vitro* culture methods¹⁸. As shown in Table 1, substantial IgA anti- $\alpha(1,3)$ dextran responses were seen only when spleen cells were obtained from old (10-17-month-old) BALB/c mice. In contrast, spleen cells from

young (4-month-old) or old (12-month-old) BALB/c (Igh-1^a) mice could be stimulated by dextran B1355 to produce IgM anti- $\alpha(1,3)$ dextran responses over a 3-5-day culture period. Similar results (not shown) were obtained in cultures using mesenteric lymph node (MLN) cells. The IgM and IgA anti-dextran B1355 antibody in spleen and MLN cultures shared idiotypic determinants with the two well-characterized anti- $\alpha(1,3)$ glucan myelomas, MOPC 104E (IgM λ_1) and J558 (IgA λ_1)¹⁹ as determined in specific inhibition studies with anti-J558 or anti-MOPC 104E antiserum.

To determine whether the absence of the IgA anti- $\alpha(1,3)$ dextran response in spleen or MLN cells from younger mice was due to cell-mediated suppression, we compared the responses in cultures containing young, old or age-mixed spleen or MLN cells. Cells from young mice did not suppress the anti-dextran response of cells from old mice (see Fig. 1).

The IgA anti- $\alpha(1,3)$ dextran response was markedly T-cell dependent (see Fig. 2). Thus, anti-Thy 1.2 and complement treatment ablated the *in vitro* IgA anti- $\alpha(1,3)$ dextran response in spleen and MLN cells and such responses were totally reconstituted by the addition of T cells.

To clarify the nature of the T-cell regulation of the IgA response, we used dextran B1355 derivatized with tri-nitrophenol (TNP)-lysine (TNP-dex)²⁰ as an *in vitro* antigen. Our experiments demonstrate that the IgA response after stimulation with TNP-dex was limited to dextran $\alpha(1,3)$ determinants. Both IgA and IgM anti- $\alpha(1,3)$ dextran responses were induced by this antigen (Fig. 3) whereas there was only an IgM response to the TNP determinant in either normal (Fig. 3a) or TNP-protein-primed (Fig. 3b) mice.

The results clearly demonstrate the age-dependence of the *in vitro* IgA anti- $\alpha(1,3)$ dextran B1355 response in BALB/c mice. Antigen-stimulated cultures from 2-8-month-old BALB/c mice did not produce significant IgA anti- $\alpha(1,3)$ glucan antibody although such cultures were competent in terms of anti-sheep red cell (SRBC) (not shown) and IgM anti- $\alpha(1,3)$ glucan responses. Note that although we did not prime the old mice used in our study, the mice may not be antigenically naive. Polyglucans are ubiquitous antigens in the mammalian digestive tract and the increase in the IgA anti- $\alpha(1,3)$ glucan response in ageing mice may be due to T- and/or B-cell priming with

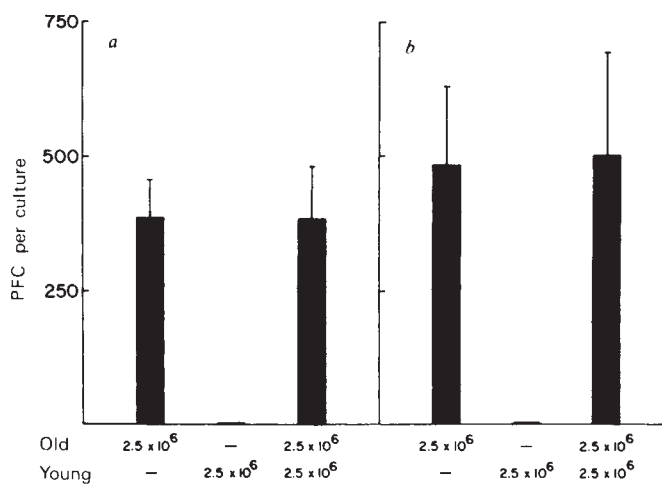


Fig. 1 Failure of lymphoid cells from young BALB/c mice to suppress IgA anti- $\alpha(1,3)$ dextran B1355 response in cultures containing lymphoid cells from old BALB/c mice. Spleen cells (a) or MLN cells (b) from 12-month-old (old) or 6-8-week-old (young) mice were cultured alone or mixed at a 1:1 ratio. Cultures containing 2.5×10^6 old cells, 2.5×10^6 young cells or 2.5×10^6 old cells and 2.5×10^6 young cells in 0.5 ml were stimulated with optimal concentrations of dextran B1355. Cultures were assayed on day 5. Data represent the mean $\pm$ s.e.m. of seven experiments (a) and four experiments (b). Culture conditions were as described in Table 1 legend.

Table 1 Age-dependence and kinetics of the *in vitro* anti- α (1,3)dextran B1355 response

Age of mice (months)	Dextran B1355 (ng per culture)	Anti-dextran response* (PFC per 10^6 cultured cells)					
		IgM			IgA		
		Day 3	Day 4	Day 5	Day 3	Day 4	Day 5
4	None	9	<2	<2	NT	NT	NT†
	0.1	67	106	16	<2	<2	<2
	1.0	78	111	32	<2	<2	<2
12	None	7	<2	<2	NT	NT	NT
	0.1	102	303	99	195	509	391
	1.0	80	259	218	199	449	289

The table shows a representative experiment in which spleen cells from 4- or 12-month-old BALB/c mice were cultured at 2.5×10^6 cells per 0.5 ml in Falcon multiwell tissue culture plates (no. 3008) and stimulated with 0.1 or 1.0 ng dextran B1355. Culture medium RPMI 1640 containing 10% fetal calf serum (FCS, lot 614 from Gibco), 2-mercaptoethanol (5×10^{-5} M), penicillin (40 U ml^{-1}) and streptomycin ($40 \mu\text{g ml}^{-1}$) was enriched with 12% cocktail¹⁶. Results are means of duplicate cultures.

* Specific anti- α (1,3) responses were assayed using palmitoyl dextran B1355 coupled to HRBC²¹. IgM and IgA anti-dextran plaques were totally inhibited by dextran B1355 but not by dextran B512 (95% α (1,6) and 5% α (1,3)glucan linkages) added to the plaque-forming cell (PFC) assay²¹. IgG responses, including IgG₃, were not detected using polyvalent and subclass-specific enhancing reagents.

† NT, not tested. In other experiments, low background levels of IgA anti-dextran PFC (that is, from 10 to 40 PFC per 10^6 cultured cells) were noted regularly in lymphoid cell suspensions derived from mice >10 months old but not in young mice.

naturally occurring environmental antigens at mucosal surfaces²¹⁻²³.

We point out that the age at which IgA responses were observed *in vitro* was considerably delayed compared with our previously reported *in vivo* results in which responses were seen between 1 and 6 months of age²¹. Because over 95% of the anti-dextran B1355 antibody *in vivo* and *in vitro* showed idiotype determinants that cross-reacted with the two well-characterized myeloma proteins J558 and MOPC104E, we conclude that both systems produce antibodies that are similar or identical. We have no convenient explanation for the differences observed *in vivo* and *in vitro*. Cell-mediated suppression does not seem to explain the lack of the *in vitro* IgA response in young mice (see Table 1), but a relative deficiency of specific T or B cell or the size (that is, molecular weight $\sim 4 \times 10^7$), structural or chemical characteristics of the molecule may explain the fact that immunization with dextran B1355 is less efficient *in vitro* than *in vivo*. In addition, differences between the *in vitro* and *in vivo* systems may depend on the microenvironment or adherent cell requirements^{24,25} for immunocompetence.

The T-dependence of the *in vitro* IgA anti- α (1,3)dextran response was striking and confirms our previous *in vivo* report²¹. Invariably, the removal of T cells from spleen or MLN populations eliminated the IgA anti- α (1,3) response and such responses were restored by the addition of T cells. Moreover, the T-dependent IgA anti- α (1,3) response did not seem to require conventional carrier-specific T-cell help⁶. Thus, with TNP-dextran as an immunogen, we routinely observed IgM and IgA anti- α (1,3)dextran responses and IgM anti-TNP responses in spleen and MLN cultures from unprimed or TNP-protein-primed mice, whereas such cultures produced no significant IgA anti-TNP response. The specificity of the response for α (1,3)glucan determinants argues against polyclonal activation by dextran in this system²⁶ or a role for IgA isotype-specific T-helper cells²⁷. However, we cannot exclude the possibility that the IgA anti- α (1,3)glucan precursor B cell that responds to T-cell help has special cell-surface determinants or properties that are lacking on normal or TNP-primed precursor B cells. We prefer the interpretation that T-cell help for the IgA anti- α (1,3)dextran response is directed to idiotype determinants on

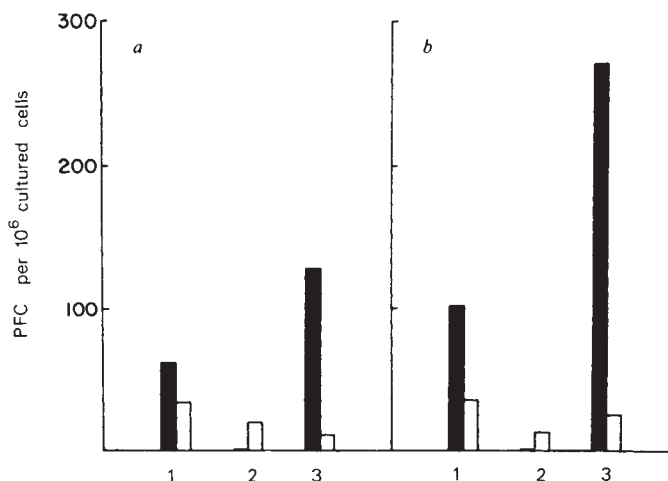


Fig. 2 Representative experiments showing the T-cell dependence of the IgA anti- α (1,3)dextran B1355 response in BALB/c mice. Cultures containing spleen cells (a) or MLN cells (b) were treated with complement alone (1), or anti-Thy 1.2 and complement (2). Additional cultures (3) were treated with anti-Thy 1.2 and complement and reconstituted with MLN which were depleted of B cells before mitomycin C treatment according to a two-step panning procedure³⁷ (a) or with mitomycin C-treated spleen cells (b). Cells from triplicate cultures were pooled and assayed on day 5 for specific IgA (solid bars) or IgM (open bars) anti- α (1,3)dextran B1355 responses. Culture conditions were as described in Table 1 legend. IgM anti-SRBC responses in parallel control cultures were totally ablated by anti-Thy 1.2 and complement treatment and restored by T cells (not shown).

anti- α (1,3)glucan antibody associated with the B cell or perhaps accessory cells, and not to α (1,3) determinants on dextran B1355.

Considerable evidence in mice substantiates the ability of antibody directed at specific immunoglobulin idiotypes to suppress or stimulate B cells²⁸⁻³². There is less evidence, however, for T-cell help directed towards idiotype determinants³³⁻³⁶. Previous studies reported that anti-idiotypic T-cell help for the B-cell response was either antigen-indepen-

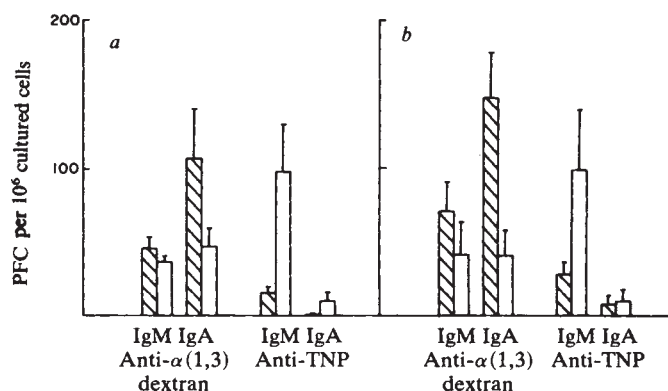


Fig. 3 Specific anti- α (1,3)dextran and anti-TNP responses in spleen or MLN cultures stimulated with TNP-dextran B1355. Cultures containing 2.5×10^6 spleen or MLN cells from 10-17-month-old unprimed BALB/c mice (a) or BALB/c mice primed three times at 1-4-month intervals with TNP-ovalbumin or TNP-keyhole limpet haemocyanin (b) were stimulated in culture with TNP-dextran B1355 (1 ng per culture, hatched bars or 10 ng per culture, open bars). Cultures were assayed 5 days later for specific IgM and IgA anti- α (1,3)dextran B1355 and anti-TNP responses. Data represent the mean $\pm$ s.e.m. of five experiments with unprimed and six experiments with TNP-primed mice. Culture conditions were as described in Table 1 legend. Control cultures from TNP-protein-primed mice stimulated with TNP on the homologous carrier yielded >400 IgA anti-TNP PFC per 10^6 cultured cells.

dent³⁵ or required two populations of T cells, one carrier-reactive and the other idiotype-specific³⁴. The striking observation in our study was the antigen-dependence and apparent lack of need for carrier-specific T-cell help for the IgA anti- $\alpha(1,3)$ response *in vitro*. Thus, T-helper cells are important in the regulation of the immune response to polysaccharide antigens such as dextran B1355. Moreover, anti-idiotypic T-helper cells may play a major part in influencing the induction and regulation of the B-cell repertoire to polysaccharide antigens.

The immune response to such antigens has been difficult to

study, partly because of the lack of a suitable *in vitro* system for examining the induction and regulation of the antibody response to such antigens. Our study demonstrates the successful application of *in vitro* culture methods to study the antigen-specific induction of antibody responses to purified dextran B1355 and seems to represent the first report of an *in vitro* antigen-induced response by dispersed cell culture to a purified polysaccharide antigen.

This work was supported by NIH grant AM. 17276, a grant from the National Foundation for Ileitis and Colitis Inc., and a grant from Nestlé, Vevey, Switzerland.

Received 2 March; accepted 15 April 1981.

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Kainate-like neurotoxicity of folates

John W. Olney, Terry A. Fuller & Taisija de Gubareff

Department of Psychiatry, Washington University School of Medicine, St Louis, Missouri 63110, USA

Kainic acid (KA) is one of the most powerful of a group of 'excitotoxic' analogues of the putative neurotransmitter glutamate (Glu) whose neurotoxicity may involve an excitatory mechanism mediated through glutamergic postsynaptic receptors^{1–8}. The finding⁹ that neural membranes have specific sites where KA binds quite firmly and that Glu inhibits such binding very weakly, however, raises the possibility that KA and Glu receptors may be separate and distinct (see also refs 10, 11). It is in any case known that the neurotoxic properties of KA and Glu are not identical. Thus, when injected into the amygdala, both Glu and KA destroy local neurones but only KA induces sustained limbic seizures and an apparently seizure-mediated pattern of extra-amygdaloid brain damage^{12–14}. Ruck *et al.*¹⁵, having recently found that the folic acid derivative, methyltetrahydrofolate (MTHF), competes powerfully for KA binding sites on rat cerebellar membranes and mimics KA in depolarizing frog spinal neurones, proposed that MTHF may be an endogenous neuromodulator with both excitatory and neurotoxic properties. We have therefore injected MTHF directly into the amygdala of the adult rat and found that at a rather high dose (300 nmol), it reproduces the specific component of KA neurotoxicity that Glu fails to reproduce, namely the limbic seizure/brain damage syndrome. We have also found that folic acid itself (pteroyl-L-glutamic acid, PGA) and one of its reduced derivatives (*N*-5-formyltetrahydrofolate, FTHF) are substantially more powerful than MTHF in reproducing this syndrome.

Fifty-five adult Harlan rats (250–300 g) were anaesthetized with ether and Nembutal (45 mg per kg) and injected stereotactically with KA (3 nmol), sodium MTHF (10–300 nmol), sodium PGA (25–150 nmol) or calcium FTHF (25–150 nmol) into the amygdala (Pellegrino and Cushman coordinates AP –0.4, L5, V9). Each agent was dissolved in sterile distilled water

immediately before use with NaOH added as needed to neutralize pH and improve solubility. Injections were delivered in volumes of 0.5–3.0 μ l over a 5-min period through a 30-gauge needle and Hamilton microsyringe. All animals were killed by perfusion fixation 4 h after injection, as by this time the neurotoxic manifestations of KA are clearly evident in histological sections^{16,17}. Brains were processed for evaluation by light and electron microscopy as described previously¹⁸.

As in earlier experiments^{12,13,19}, KA injection (3 nmol) caused staring, mouth movements, head bobbing, eye blinking, wet dog shakes, sialorrhoea and limbic seizures which, in some animals, could be described as 'status' limbic seizures. All KA-injected animals ($n = 6$) had a conspicuous acute neurotoxic reaction at the local injection site, consisting of massive dilatation of neuronal dendrites, swelling of astroglia and either oedematous swelling or vacuolization and dark cell degeneration of neuronal somata. The local reaction was typically most severe in the region immediately surrounding the injection site but many amygdaloid neurones further from the injection were also damaged, as were neurones in the piriform, entorhinal and temporoparietal cortices, the hippocampus, lateral septum and certain thalamic nuclei. In some animals all these brain regions were involved but in others only a partial syndrome developed. An apparent 'mirror' focus of acute neuronal degeneration was evident in the contralateral amygdala of one animal. This disseminated pattern of damage in KA-treated animals, which several authors have attributed to a seizure mechanism^{12,13,19–22}, is illustrated in refs 17, 21, 22.

When MTHF was injected into the amygdala at 10, 30, 60, 100 and 300 nmol, tissue and behavioural pathology was observed only at the highest dose. Of four animals injected with this dose, three were affected; each displayed periodic KA-type seizures over a 1–2 h period and had mild brain lesions primarily involving the piriform cortex (Fig. 1a, b). Amygdaloid neurones were less consistently damaged and the tissue zone immediately surrounding the amygdala injection site was sometimes totally free from damage.

PGA and FTHF were both injected into the amygdala at 25, 50, 100 and 150 nmol (four animals at each dose of each agent) and every dose of each compound reproduced a KA-type seizure/brain damage syndrome. At 25 nmol, the seizures

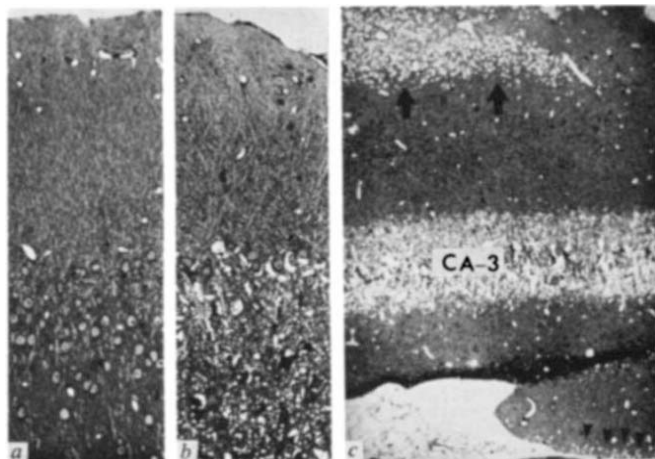


Fig. 1 *a, b*, The adult rat piriform cortex following injection of 60 nmol (*a*) or 300 nmol (*b*) of MTHF into the amygdala. The piriform cortex of *a* appears normal and this animal exhibited no behavioural pathology. The piriform cortex of *b* displays the typical acute cytopathological changes seen in animals following an intracerebral injection of KA (illustrated in refs 16, 18, 21); this animal had frequent KA-type seizures for 2 h ($\times 106$). *c*, The adult rat hippocampus following injection of 100 nmol PGA into the amygdala; the pattern of acute cytopathological changes is identical to that described and illustrated elsewhere²¹ in the hippocampus of KA-treated rats. Note the extreme oedematous changes in dendritic and glial structures which form a band of rarified tissue in the CA-3 pyramidal layer and the dilated distal CA-3 apical dendrites (arrows) and distal dentate granule dendrites (arrowheads) ($\times 60$).

induced by either agent were relatively mild and the brain damage largely confined to the olfactory cortex, although cytopathological changes were also sometimes detected both at the local amygdaloid injection site and in several remote brain regions. At 100 and 150 nmol, animals typically manifested severe KA-type behavioural symptoms including sustained limbic seizures, and their brains exhibited the full pattern of cytopathological changes associated with KA treatment (Fig. 1c). However, the tissue reaction at the amygdala injection site was not identical to that induced by KA in that damage to neuronal groups adjacent to the injection site was sometimes either mild or nonexistent.

When KA (2–10 nmol) is injected into the rat striatum, it destroys many local neurones²³ and, in addition, induces sustained seizures which are accompanied by the disseminated pattern of apparently seizure-mediated brain damage associated with KA injection into the amygdala^{17,24}. Intra-striatal injection of Glu (500–1,000 nmol) or the potent linear aspartate analogue *N*-methyl aspartate (40–80 nmol) also results in acute destruction of striatal neurones, but is not accompanied by either seizures or disseminated (distant) brain damage^{7,13,24}. To discover whether folates reproduce both the local and distant neurotoxic actions of KA, we injected PGA (50 and 100 nmol) into the striatum of halothane-anaesthetized rats; all animals ($n=6$) manifested a full repertoire of KA-type symptoms, including frequent seizures, and all displayed the KA pattern of distant brain damage, but none sustained damage at the local striatal injection site²⁹. We tentatively conclude, therefore, that PGA and FTHF are partial KA-like neurotoxins which faithfully reproduce the distant (seizure-linked) pattern of KA-type damage while failing to reproduce the powerful local (direct) brain-damaging action of KA. The present finding of folate-induced damage to the amygdala can possibly be attributed to a seizure mechanism, as amygdala neurones are a vulnerable part of the excitatory limbic circuits within which this type of damage occurs. Additional studies are needed to clarify whether folates are totally without local neurotoxicity or merely display local neurotoxic manifestations more weakly than distant ones, and

whether separate mechanisms underlie the seizure-linked neurotoxic properties shared by KA and folates and the direct neurone-necrotizing properties shared by KA and Glu linear analogues.

It is believed that all the metabolic functions of folates are mediated by reduced tetrahydrofolate (THF) forms of PGA and that PGA itself is metabolically inactive. Because PGA was a much more active neurotoxin in our experiments than was its reduced derivative, MTHF, it seems unlikely that PGA neurotoxic activity is dependent on its conversion *in vivo* to MTHF. To explore the possibility that PGA activity depends on conversion to FTHF, we injected dihydrofolate (DHF) into the amygdala. As this compound is an intermediary metabolite in the PGA $\rightarrow$ FTHF pathway, it should convert *in vivo* to FTHF as readily as does PGA and, therefore, should be at least as active as PGA. However, we found DHF, like MTHF, to be quite weak in reproducing KA-like neurotoxicity¹⁴. Moreover, we injected amethopterin, an irreversible blocker of DHF reductase, into the amygdala 15 min before PGA injection¹⁴; although amethopterin presumably blocks conversion of PGA to any of its reduced forms, it did not decrease or prevent the expression of KA-like neurotoxicity. Thus, we suspect that the PGA molecule itself may have KA-like neurotoxic activity, and that FTHF independently has such activity.

A report²⁵ that PGA is not taken up by brain tissue slices is of interest here because the enzymatic conversion of PGA to reduced derivatives probably occurs intracellularly and cannot do so if PGA is not taken up by brain cells. Thus, injection of PGA into brain might be expected to result in its extracellular accumulation, allowing maximum opportunity for it to interact with neuromodulatory folate receptors, if any exist, on the external surfaces of central neurones. The efficient intracellular transport (and thus inactivation) of DHF and MTHF could explain their lower activity. This possibility, and the intriguing alternative that PGA and FTHF differ from other folates in having subtle structural features in common with KA, should be further investigated.

Others have shown that folates are convulsants^{26,27}, but it has not previously been demonstrated that they are also powerful brain damaging agents. Since folates are naturally present in the central nervous system and the seizure-related pattern of brain damage we describe here in folate-treated rats conforms closely to that frequently observed in autopsied brains of human epileptics²⁸, a possible role for folates in human epilepsy and epileptic brain damage warrants consideration.

KA, MTHF, PGA and FTHF were obtained from Sigma. This work was supported in part by grants from the USPHS, the Wills Foundation and Research Career Scientist Award MH-38894 (to J.W.O.).

Received 2 March; accepted 1 May 1981.

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Novel leukaemogenic retroviruses isolated from cell line derived from spontaneous AKR tumour

Finn Skou Pedersen*, Robert L. Crowther†, Donald Y. Tenney†, Andreas M. Reimold† & William A. Haseltine†

* Department of Molecular Biology, University of Aarhus, 8000 Aarhus C, Denmark

† Sidney Farber Cancer Institute, Department of Pathology, Harvard Medical School and Department of Microbiology, Harvard School of Public Health, Boston, Massachusetts 02115, USA

Viruses isolated from leukaemic and preleukaemic tissues of the AKR mouse differ in their ability to induce thymic lymphomas. The Gross passage A virus, which originates from the thymus of a leukaemic AKR mouse¹, and the MCF viruses, isolated from preleukaemic and leukaemic AKR tissues^{2,3}, accelerate the onset of disease on injection into newborn AKR mice^{3,4} whereas the endogenous ecotropic Akv virus, which is expressed in AKR mice from mid-gestation onward, is nonleukaemogenic^{3,5,6}. What are the structural features of the genome that account for the leukaemogenic activity of these viruses? The observation that the gp70 envelope glycoprotein of the leukaemogenic MCF virus contain ecotropic and xenotropic regions^{7–9} raised the possibility that the structure of the gp70 gene was the major determinant of leukaemogenic activity². However, a cloned, *in vitro* passaged, leukaemogenic isolate of the Gross A virus very closely resembles that of the nonleukaemogenic Akv virus in the *gag-pol* and gp70 coding regions of the genome¹⁰. Most of the differences in structure of the Gross A and Akv viruses are located within the 3'-terminal 1,000 nucleotides—this raises the possibility that sequences outside the gp70 gene which are proximal to the 3' end of the genome encode determinants of leukaemogenic activity. Due to the complex passage history of the Gross A virus we sought to isolate further leukaemogenic AKR viruses that had not been passaged in other animals to determine whether or not they resembled the Gross A virus. Here we report the isolation and characterization of new leukaemogenic viruses from a lymphoid cell line derived from a spontaneous AKR tumour. These isolates resemble the Gross A virus in the 3' region of the genome. Our results suggest that this region encodes determinants of leukaemogenic potency and that major changes in the envelope glycoprotein are not necessary for the leukaemogenic activity of these viruses.

The source of virus was the SL3 cell line, established from a spontaneous tumour of an AKR mouse⁵, that produces a low titre of an ecotropic, N-tropic, XC[−] virus^{5,6}. To obtain clonal isolates of this virus, the cell-free supernatant of the SL3 cell line was used to infect a culture of NIH 3T3 cells. Viruses were cloned from the supernatant of the infected culture by two rounds of end point dilution. For each round of cloning, less than one in five wells contained virus at the end point. Two viruses, SL3-1 and SL3-2, were obtained in one such experiment; the SL3-3 virus was obtained in a separate but similar experiment.

The biological properties of the cloned and uncloned SL3 were determined (Table 1). All viruses obtained from the SL3 cell line are ecotropic and N-tropic. The SL3-1 and SL3-3

viruses are positive in the XC fusion test, whereas the SL3-2 virus is not. The ratio of infectious virus to reverse transcriptase activity is much higher for virus grown in NIH 3T3 cells than for virus produced by the SL3 cell line.

The cloned SL3 isolates retain potent leukaemogenic activity (see Table 1): injection of these viruses into newborn AKR mice results in 100% incidence of thymic lymphoma with an average latent period of ~80 days. Mock-inoculated controls do not develop disease before 220 days. The pathology of the accelerated disease is indistinguishable from the spontaneous AKR or Gross passage A virus-induced thymic lymphoma. The cloned SL3-2 and SL3-3 isolates also induce rapid leukaemia in C3H₁₀₁/Bi mice (100% incidence, mean latent period of 100 days). Normally, these animals have a negligible incidence of thymic leukaemia. Our preliminary results indicate that the SL3-3 isolate also induces rapid thymic leukaemia in NSF-N mice—these animals show a pathology typical of Gross passage A-induced disease.

The structure of the SL3 viral RNAs was analysed using high resolution methods for analysis of RNase T₁-resistant oligonucleotides^{11,12}. Fingerprints of 70S RNAs of virus produced by the uncloned SL3 virus grown on NIH 3T3 cells and the cloned isolates are shown in Fig. 1, together with schematic interpretations. The fingerprint of the virus produced by the SL3 cell line itself is indistinguishable from that of the uncloned SL3 virus grown on NIH 3T3 cells. The schematic interpretations are based on co-electrophoresis of labelled oligonucleotides with those of the Akv virus, and on complete or partial sequence analysis of each numbered oligonucleotide. The fingerprints of the cloned virus indicate that they are biochemically pure and the molar yield of the unique oligonucleotides is close to unity in each isolate. The fingerprints of the cloned viruses do not change on subsequent cloning or passage of these isolates. The order of the unique oligonucleotides along the genome was determined (see Table 2). The sequence of the oligonucleotides of the SL3 viruses that are not present in the fingerprint of the Akv virus were also determined (see Table 3).

The map of Table 2 shows that oligonucleotides 208B and 36B are located near the 3' terminus of the SL3 viruses. It is striking that identical oligonucleotides are also found in the same relative positions in the leukaemogenic Gross A/NIH virus¹⁰. The sequence of oligonucleotides 208B and 36B differ from sequences in the Akv virus by one or two base changes; except for these two oligonucleotides and a few in the *gag-pol* region of the genome, the SL3-1 and SL3-3 viruses are identical to the Akv virus within the limits of resolution of the fingerprinting method, yet the SL3 viruses are potent leukaemogenic agents. Evidently, slight changes in the genome structure can affect the leukaemogenic activity of AKR viruses.

The SL3-2 virus differs from those of SL3-1 and SL3-3 cell lines in the region that encodes the gp70 protein. Most of this region is substituted with a heterologous sequence. One explanation for the isolation of two different viruses from the same cell line is that the SL3 cell produces a mixture of two viruses that have a large part of the genome that is identical but differ in the gp70 gene. The observation that, in the uncloned viruses, the oligonucleotides derived from the gp70 region of the genome are present in half the molar yield of the other oligonucleotides in the genome supports this notion (data not shown).

We have also studied viruses produced by eight other lymphoid tumour cell lines of AKR origin. Four of these produce mixtures of virus identical to those produced by the SL3 cell line and which also have the changes in the *gag-pol* and *env* regions noted here. Comparison of these viruses will be the subject of a separate communication.

Some of the gp70 oligonucleotides of the SL3-2 virus are identical to sequences found in fingerprints of a xenotropic virus chemically induced from AKR fibroblasts¹³ (see Table 3). As is the case for MCF viruses isolated from AKR tissues, substitution of xenotropic virus-related sequences within gp70 correlates with the XC[−] phenotype of the SL3-2 virus. Unlike the

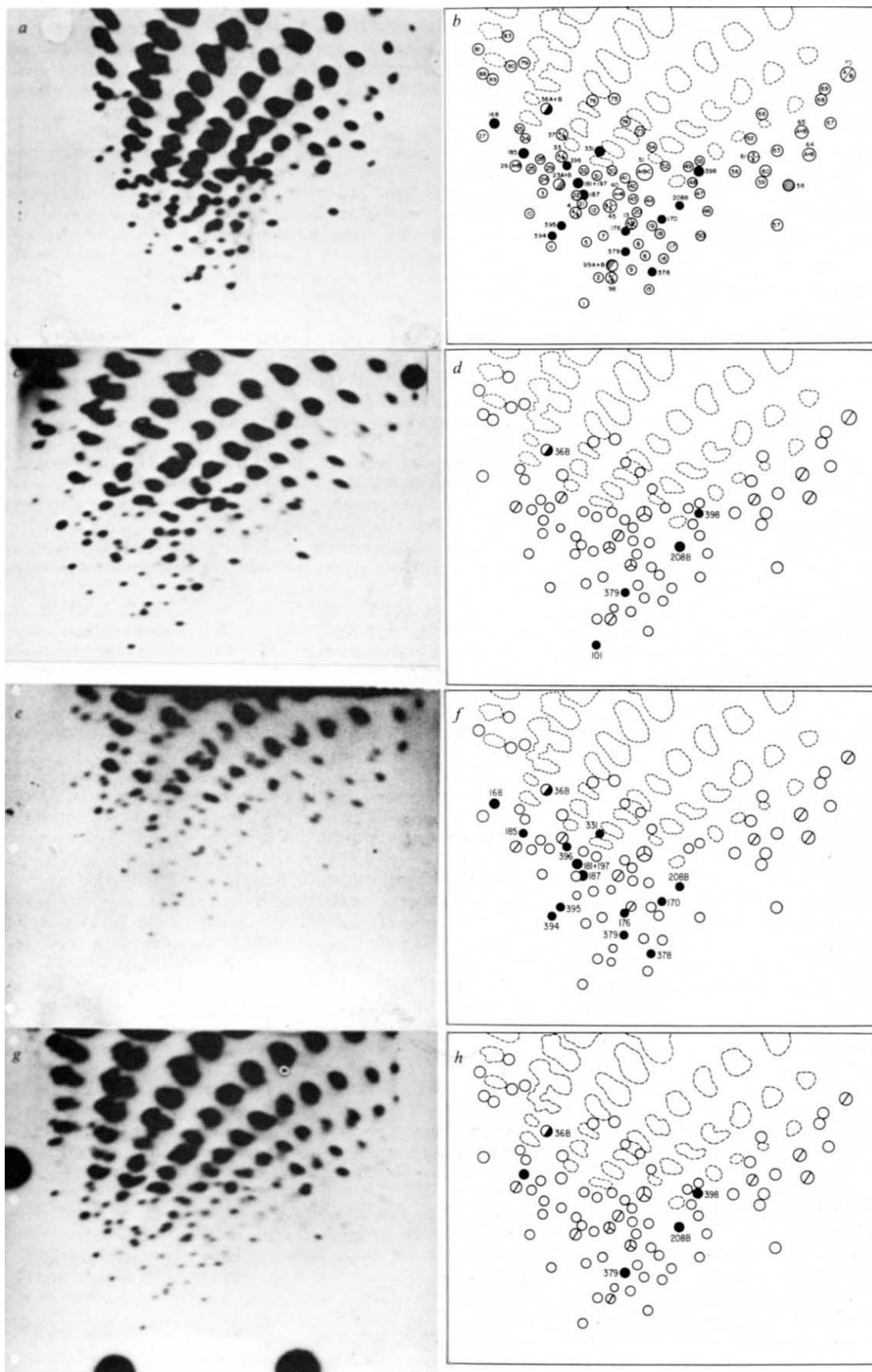


Fig. 1 RNase T_1 fingerprint analysis of SL3 viruses. Viral 70S and 35S RNAs were prepared from cell-free supernatants as described elsewhere^{11,12}. The schematic interpretations are based on (1) analysis of mixtures of labelled oligonucleotides of each viral RNA with those of the Akv virus and; (2) complete or partial sequence analysis of each numbered oligonucleotide in the fingerprints of each virus. *a, b*, 70S RNA of the uncloned SL3 virus propagated on NIH 3T3 cells. The fingerprints of the virus produced by a subline of the SL3 cell line, and virus produced by two other NIH 3T3 cultures infected with the uncloned SL3 virus were indistinguishable from this fingerprint, shown in *a*. Open circles represent oligonucleotides common to the Akv and SL3 viruses. The Akv virus used for this comparison was derived from the same mouse colony that gave rise to the SL3 cell line from (from Esther Hays, University of California, Los Angeles). Solid circles represent oligonucleotides present in the fingerprint of the SL3 virus that are not present in the fingerprint of the Akv virus. Cross-hatched circles represent oligonucleotides characteristic of the Akv genome that are absent altogether from the fingerprint of the SL3 virus. The numbering of the Akv oligonucleotides is as described previously¹². The numbering of oligonucleotides 208B and 36B is the same as that assigned to the Gross A oligonucleotides which have identical sequence¹⁰. *c, d*, 70S RNA of the SL3-1 isolate. Open circles represent oligonucleotides shared with the Akv virus; solid numbered circles represent oligonucleotides that are not found in the fingerprint of the Akv virus. *e, f*, 35S RNA of the SL3-2 isolate; symbols are the same as for *d*. *g, h*, 70S RNA of the SL3-3 isolate; symbols are the same as for *d*. Although not indicated in the schematic drawings, the same Akv-specific oligonucleotides that are missing from the fingerprints of uncloned SL3 virus are also missing from the fingerprints of the cloned derivatives. The molar yields of each of the unique oligonucleotides of each fingerprint were determined in two independent experiments by counting gel fragments corresponding to the position of the oligonucleotides. The molar yield of each of the unique oligonucleotides was the same, within experimental error, in the fingerprints of the cloned viruses. For the uncloned SL3 virus, the molar yield of the oligonucleotides in the gp70 region of genome (see Table 2) was half that of the other oligonucleotides.

AKR-MCF viruses, the gp70 substitution in SL3-2 apparently does not result in an extended host range. More important, the gp70 substitution in SL3-2 is not essential for the leukaemogenic potential of the virus, as the SL3-1 and SL3-3 viruses which lack the substitution are also potent leukaemogenic agents.

What functions relevant to leukaemogenic activity may be

altered in the SL3 viruses? All the SL3 isolates differ slightly from the Akv virus in the *gag-pol* region of the genome. It is possible to argue that these differences correlate with oncogenic potential. However, the Gross A/NIH virus also contains changes in this region of the genome, but these sequences are not the same as those present in the SL3 viruses. Therefore, we consider it more likely that the variant 3' region of the genome

Table 1 Biological properties of the SL3 viruses

Virus	NIH3T3	BALB/c	CCL64	8155	FLF ₃	c.p.m. per Cell (RT)	IU/ml ⁻¹ end point	S+L - PFU/ml ⁻¹	XC PFU ml ⁻¹	Leukaemogenicity acceleration days post-inoculation	Induction in C3H ₁ /Bi No induction
Akv	+	-	-	-	-	2.3×10^{-1}	1×10^7	2×10^5	1×10^5	No acceleration	No induction
Gross A/NIH	+	-	-	-	-	2.5×10^{-1}	2×10^7	2.5×10^5	3×10^5	102-146 (ref. 10)	135-220 (ref. 4)
SL3	+	-	-	-	-	2.5×10^{-3}	1	0	0	80-100 (ref. 6)	NT
SL3/NIH	+	-	-	-	-	3.3×10^{-1}	2×10^6	3×10^3	60	69-104	NT
SL3-1	+	-	-	-	-	1.4×10^{-1}	1×10^5	3×10^3	7×10^3	120-130	NT
SL3-2	+	-	-	-	-	2.6×10^{-1}	1×10^4	2.25×10^5	0	69-105	84-114
SL3-3	+	-	-	-	-	2.5×10^{-1}	1×10^6	3.5×10^4	6×10^4	61-82	73-117
AKR-6	-	-	+	+	+	2.5×10^{-1}	1.10^6	NT	NT	NT	NT
MCF-247	+	-	+	-	-	3.5×10^{-1}	NT	NT	NT	90-155 (refs 3, 21)	NT

The biological properties of the viruses were determined using standard methods. The viruses used were Akv and Gross A/NIH described elsewhere¹⁰⁻¹²; AKR MCF-247; a xenotropic virus, AKR-6, derived from tissues of an AKR mouse propagated on dog 8155 cells, and the SL3 viruses described in the text. The same preparation of each virus was used for all tests. The ability of the viruses to grow on mouse NIH/3T3 and BALB/c cells, mink CCL64 cells, dog 8155 cells, and cat FLF₃ cells was determined by assay of reverse transcriptase activity in cell-free supernatants. The reverse transcriptase activity produced per cell was determined by dividing the c.p.m. incorporated by the virus in 1 ml of culture fluid in a standard reverse transcriptase assay by the number of cells per ml (in the case of suspension cultures) or the number of cells per plate per ml of supernatant (for adherent cells). The end point titre (in Infectious Units, IU) was determined in NIH3T3 cells. The S+L- and XC fusion assays are those described elsewhere^{22,23}. PFU, plaque-forming units. The leukaemogenic activity of each virus was determined by inoculation of 0.1 ml of cell-free supernatant intraperitoneally into 2-4 day-old AKR and C3H₁/Bi mice. Mockinjected littermates served as controls in all cases. Experimentally induced acceleration of disease in AKR mice was clearly a separate event from that of spontaneous disease in control littermates. All eight animals injected with the uncloned SL3 virus grown on NIH3T3 cells (SL3/NIH) developed leukaemia in 69-104 days with an average onset of 87 days, whereas seven control littermates did not develop disease before 220 days. All five animals injected with the SL3-1 virus developed disease at 120-141 days, average 124 days post-inoculation, whereas three uninoculated littermates did not develop disease before 220 days. All five animals injected with the SL3-2 virus developed thymic lymphomas between 82 and 141 days (average 92 days), whereas eight control littermates remained disease-free for >230 days. All eight animals injected with the SL3-3 virus developed disease between 61 and 82 days whereas six control littermates remained disease-free at 230 days post-inoculation. Induction of disease in the low spontaneous incidence strain C3H₁/Bi demonstrated an equivalent potency in these mice for two of the viral isolates. All eight animals injected with SL3-2 developed thymoma between 84 and 114 days (average 99 days), and nine animals injected with SL3-3 developed disease between 73 and 117 days (mean 100 days post-inoculation). Thymic lymphoma was diagnosed by autopsy of sick animals except in a few rare cases in which animals died and were then autopsied. NT, not tested.

Table 2 Oligonucleotide maps of the SL3 viruses

Akv / NIH										
Gross A / NIH	2	12		13C					55B	f
SL3-3	58		238 29		99A				55B	f
SL3-1	58	1	238		99A				55B	f
SL3-2	58		238		99A	88 46	17 20 56 21 13A 11 10	24 9 4A 23A 8 47	55B	f
Akv / NIH										
Gross A / NIH		201 204		206				202	211	208 208 A 36B
SL3-3				379						208 B 36B
SL3-1		101		379						208 B 36B
SL3-2				379		168 170 (176 181 185 187 331 396) 394 395/197 378				208 B 36B

The order of the large T₁ oligonucleotides of the genome of the SL3-2 virus was determined as described elsewhere¹⁰. The map order of the Akv and Gross A/NIH viruses are those described elsewhere¹⁰. The map order of the oligonucleotides of the SL3-1 and SL3-3 viruses are inferred by comparison with the maps of the Akv, Gross A/NIH and SL3-2 viruses. The upper half represents oligonucleotides, present in Akv, which are missing from these viruses, the lower half represents the positions of oligonucleotides which are not present in Akv. For the Akv genome, there are a total of 34 oligonucleotides within the *gag-pol* region, 16 within gp70, 9 within p15E, and 6 within U₃. The assignment of oligonucleotides 88 and 46 to the *env* region of Akv, Gross A, SL3-1, and SL3-3 is further supported by alignment of these sequences with the protein sequence of the *env* gp70 proteins of the Moloney and Rauscher murine leukaemia viruses²⁴. This alignment is indicated below

1
Ala-Ala-Pro-Gly-Ser-Ser-Pro-His-Glu-Val-Tyr-Asn-Ile-Thr-Trp
GC CCC CAC CAG GUU UUU AAC CUC ACC UG
* *
1- 88 -1- 46 -1

An asterisk indicates positions in which the oligonucleotide sequence differs from that predicted by the amino acid sequence. The alignment of oligonucleotides with p15E-R and U₃ is further supported by DNA sequences of the Moloney leukaemia¹⁵ and Akv viruses (W. Herr, personal communication and J. Lenz, R. Crowther, A. Straceski and W. Haseltine, unpublished data). In addition to those shown, the Gross A/NIH virus possesses oligonucleotides numbered 205, 207, 213 and 215, which, by fingerprint analysis, map within the extreme 3'-terminal regions. Their precise locations relative to other oligonucleotides within p15E and U₃ are unknown.

Identification of *E. coli* *uvrC* protein

George H. Yoakum & Lawrence Grossman

Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, Maryland 21205, USA

The excision of pyrimidine dimers from DNA is one of the major mechanisms of repair involving an initial endonucleolytic step followed by excision, resynthesis and ligation¹. The enzymatic mechanism for endonucleolytic cleavage of UV light-damaged DNA by the *Micrococcus luteus*² and *Escherichia coli* bacteriophage T4 (ref. 3) dimer-specific endonucleases involves two steps: cleavage of the *N*-glycosylic bond of the 5'-pyrimidine moiety of the pyrimidine dimer, followed by cleavage of the phosphodiester bond 3' to the apyrimidinic site produced by the first step. Although *E. coli* requires functional *uvrA*, *uvrB* and *uvrC* genes to repair UV light-damaged DNA⁴, and enzymatic cleavage of damaged DNA requires the concerted action of at least three proteins⁵⁻⁸, the mechanism of catalysis by which the *uvr*-endonucleolytic system cleaves UV light-damaged DNA is unclear. We have constructed hybrid multicopy plasmids containing the *uvr* genes of *E. coli*⁹ to study the expression of amplified *uvr* genes. We have physically mapped *uvrC* on plasmid pGHY4211 (previously referred to as pGY4211 (ref. 9)) by interruption with the $\gamma\delta$ sequence of F⁺ as described by Guyer¹⁰, and determined the minimum size for the *uvrC* gene to be 1.45 kilobase pairs. SDS-gel electrophoresis of radioisotopically labelled plasmid proteins indicates that the *uvrC*⁺ gene product is a polypeptide of molecular weight (MW) 68,000. We estimate that wild-type *E. coli* may have as few as 10 copies of *uvrC* polypeptide per cell.

We have previously described a set of plasmids, consisting of pBR322 and a 3.4-kilobase pair *Pst*I fragment of *E. coli* K12 DNA, which complements the *uvrC34* mutation in either orientation relative to pBR322 (pGHY4211, pGHY3233)⁹. To study the role of *uvrC* in the *uvr* repair system of *E. coli* we have physically mapped the *uvrC* gene, and identified the *uvrC* protein by SDS-gel electrophoresis of plasmid proteins labelled by the maxi-cell¹¹ method.

Inactivation mapping of cloned genes has proved useful in determining the physical size, direction of transcription and identification of gene products¹²⁻¹⁵. The $\gamma\delta$ -insertion sequence of F⁺ was used to identify the *uvrA* and *uvrB* gene products in a similar manner^{16,17}. This insertion sequence integrates into DNA by a *recA*-independent mechanism¹⁰, and DNA sequencing indicates that termination signals occur in five of the six possible reading frames near the ends of the $\gamma\delta$ sequence (M. Guyer, personal communication). Thus, insertion of the $\gamma\delta$ sequence into a structural gene terminates translation in almost all cases. The F⁺ *recA56* streptomycin-sensitive (Str^s) strain (MG1063) used here to isolate $\gamma\delta$ insertions in plasmid pGHY4211 was provided by M. Guyer¹⁰, and was transformed¹⁸ with pGHY4211. Transconjugants of pGHY4211 containing $\gamma\delta$ insertions were isolated by mating tetracycline-resistant (Tet^r) MG1063 carrying pGHY4211 with an F⁻ *recA56 uvrC34 rpsL* strain (SR57, Kendrick Smith). To select recipient cells carrying pGHY4211 with $\gamma\delta$ insertions, cultures were plated on Luria agar containing 10 $\mu\text{g ml}^{-1}$ tetracycline and 50 $\mu\text{g ml}^{-1}$ streptomycin, and colonies were tested for Tet^r and Str^r (ref. 10). About 200 Tet^r Str^r colonies were tested for UV light sensitivity as described elsewhere⁹. Several colonies carrying plasmids unable to complement the *uvrC34* mutation of SR57 (*recA56 uvrC34*) were selected for further study, and small amounts of plasmid DNA isolated by a rapid isolation procedure¹⁹. The *recA56 uvrC34* double mutant (SR57) was transformed with the plasmid DNAs isolated above, screened

for Tet^r, Str^r and UV light sensitivity, then stored at -20 °C in glycerol cultures for later use in protein labelling experiments.

The site of integration was determined for 10 $\gamma\delta$ insertions in pGHY4211 which inactivate the *uvrC*⁺ function of this plasmid, by measuring the size of restriction fragments after digestion with *Eco*RI or *Bam*HI. pGHY4211 contains only one *Eco*RI and one *Bam*HI site in the pBR322 portion²⁰ of this 7.76-kilobase pair plasmid⁹. Therefore, the *Eco*RI site located at 0.9 kilobase pair from the γ end and the *Bam*HI site at 0.4 kilobase pair from the δ end of the insertion sequence¹⁰ allow an unequivocal location of the site of $\gamma\delta$ insertion and its relative orientation, by determining the size of the *Eco*RI and *Bam*HI restriction fragments on agarose gels. Mapping data yielded the locations of $\gamma\delta$ insertions (shown in Fig. 1), indicating that the minimum size for the *uvrC* gene transcription unit is 1.45 kilobase pairs. This is enough genetic information to encode an average polypeptide of MW 56,000.

SDS-polyacrylamide gel electrophoresis²¹ of ³⁵S-Met-labelled proteins encoded by $\gamma\delta$ -inactivated pGHY4211 plasmids, reveals that a 68,000-MW polypeptide is missing in each case (Fig. 2b). This 68,000-MW polypeptide is present among the plasmid-encoded products of pGHY4211 and pGHY3233 (Fig. 2a, b) which contain the *uvrC*⁺ 3.4-kilobase pair *Pst*I fragment in both orientations relative to pBR322. As the *uvrC* gene transcription unit in pGHY4211 maps between 0.85 and 2.3 kilobase pairs (Fig. 1), and a 68,000-MW polypeptide is missing from the plasmid-encoded products of each $\gamma\delta$ -inactivated plasmid, these data indicate that the 68,000-MW polypeptide is encoded by *uvrC*. However, these data do not preclude the possible disappearance of a low-molecular weight polypeptide, fortuitously similar to a low-molecular weight polypeptide among $\gamma\delta$ plasmid-encoded background bands in Fig. 2. Although identification of the *uvrC* gene product must also be established by conventional protein purification methods, the 68,000 molecular weight observed here is in the same range suggested for *uvrC* from complementation activity in *E. coli* extracts⁷.

Additional evidence that the 68,000-MW polypeptide is encoded by the *uvrC* gene was obtained by *in vitro* mutagenesis of pGHY3233. Mutations in the *uvrC*⁺ gene of pGY3233 were isolated by irradiation of plasmid DNA with various doses of UV light and transformation¹⁸ of irradiated plasmid DNA into *rec*⁺ *uvrC34* AB1884 (ref. 4). About 800 Tet^r transformants were tested for Tet^r and UV light sensitivity as previously described⁹. From these, two colonies were found which carried Tet^r plasmids that were unable to complement the *uvrC34* mutation. After isolation of a small amount of plasmid DNA, SR57 *recA56 uvrC34* was transformed with pGHY7077 and pGHY7064 and cultures tested for Tet^r and UV light sensitivity and placed in storage as described above. pGHY7077 and

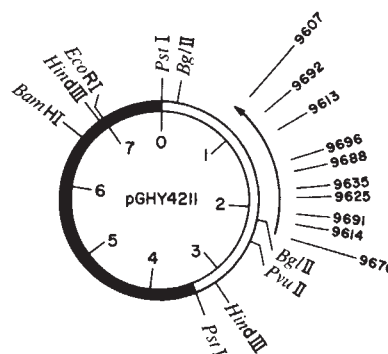


Fig. 1 $\gamma\delta$ insertion map of the *uvrC* gene in plasmid pGHY4211 including the minimum physical size of the *uvrC* gene transcription unit, and the most probable direction of transcription. The site of $\gamma\delta$ integration in pGHY4211 was determined by restriction mapping as described in the text. The most distant sites of integration which inactivate the *uvrC*⁺ function of pGHY4211 are at 0.85 kilobase pair (9607) and 2.3 kilobase pair (9670), indicating a minimum size of 1.45 kilobase pair for *uvrC*.

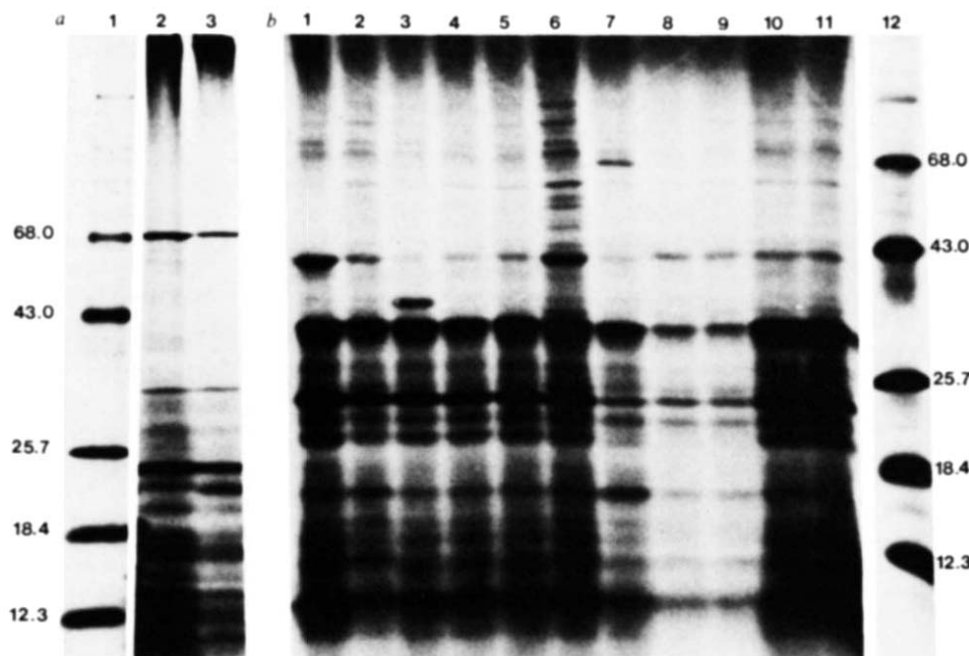


Fig. 2 The ^{35}S -Met-labelled protein products of various plasmids were visualized by fluorography of maxi-cell extracts resolved on 10–20% polyacrylamide gradient 2% SDS gels by electrophoresis, as described in the text. The first lane in *a* contains ^{14}C -labelled protein standards obtained from Bethesda Research Laboratories. Lane 2 contains ^{35}S -Met-proteins from *uvrC*⁺ pGHY3233 (ref. 9); lane 3, ^{35}S -Met-labelled proteins from pGHY4211. *b*, Fluorography of a similar polyacrylamide gel used to resolve the ^{35}S -Met-labelled proteins encoded by the plasmid indicated for each lane. The electrophoretic mobility of protein ^{14}C -labelled standards is indicated in the margin. Lane 1, $\gamma\delta$ -9607; lane 2, $\gamma\delta$ -9866; 3, $\gamma\delta$ -9613; 4, $\gamma\delta$ -9614; 5, $\gamma\delta$ -9625; 6, $\gamma\delta$ -9670; 7, pGHY4211 (*uvrC*⁺); 8, pGHY7064 (*uvrC*[−] mutation); 9, pGHY7077 (*uvrC*[−] mutation); 10, $\gamma\delta$ -9691; 11, $\gamma\delta$ -9696. The appearance of a number of bands in lane 6 ($\gamma\delta$ -9670) could have resulted from an increase in background due to a low level of contamination for this extract.

pGHY7064 plasmid DNAs were digested with *Pst*I and *Hind*III and compared with pGHY3233 by agarose gel electrophoresis (data not shown). Both pGHY7077 and pGHY7064 contain the 3.4-kilobase pair *Pst*I fragment in the same orientation as their *uvrC*⁺ counterpart, pGHY3233 (ref. 9). Radioisotopic labelling of plasmid-encoded proteins from *uvrC*[−] pGHY7077 and pGHY7064 derived by *in vitro* UV light mutagenesis lack a 68,000-MW polypeptide (Fig. 2*b*). This confirms the conclusion drawn from the $\gamma\delta$ -inactivation experiment that the 68,000-MW polypeptide is encoded by the *uvrC* gene.

The most probable orientation of *uvrC* transcription was deduced from the polypeptide products and map position of $\gamma\delta$ -9613—this maps at 1.275 kilobase pairs (Fig. 1), and produces a unique polypeptide of MW 35,000 (Fig. 2*b*, lane 3). The appearance of this polypeptide among the ^{35}S -Met-labelled products of a plasmid that is unable to produce the 68,000-MW polypeptide and inactivated for *uvrC* complementation, suggests that the 35,000-MW polypeptide is a partial product of *uvrC*. Because the 1-kilobase pair distance between 9613 and 9670 is adequate to encode an average polypeptide of MW ~38,300, and the 420 base pairs from 9613 to 9607 could only encode ~16,200 MW, the position of 9613 and the 35,000-MW polypeptide are most consistent with a starting point for the *uvrC* gene close to 9670, with transcription proceeding towards 9607 (Fig. 1). In addition, we found that $\gamma\delta$ -9692 produces a similar truncated polypeptide (data not shown) to that produced by $\gamma\delta$ -9613, supporting the orientation of transcription deduced from the distribution of $\gamma\delta$ inserts which inactivate the *uvrC* gene and the 35,000-MW truncated product from $\gamma\delta$ -9613.

To estimate the approximate number of *uvrC* polypeptide chains synthesized in wild-type *E. coli*, we compared the densitometric intensity of the *uvrC* and *ssb* bands from the labelled protein products of a *uvrC*⁺ *ssb*⁺ *uvrA*⁺ composite plasmid (pGHY4610)⁹. After labelling maxi-cell extracts of pGHY4610 (*uvrA*⁺ *ssb*⁺ *uvrC*⁺)⁹ with ^{14}C -amino acid mixtures consisting of 20 amino acids present in similar specific activity, the labelled products were visualized by SDS-polyacrylamide gel electrophoresis and fluorography as described above. Autoradiographic films were exposed for various lengths of time and densitometric scans made. A comparison of the *ssb*⁺ band, present in 1,200 polypeptide chains per *E. coli* cell²², with the *uvrC*⁺ band, indicates that *uvrC* may be present in as few as 10 copies per cell (Fig. 3).

The *uvrC*⁺ protein of *E. coli* is identified as a 68,000-MW protein on SDS-polyacrylamide gels. This protein is required for

ATP-dependent incision of DNA containing pyrimidine dimers⁷ and psoralen cross-links²³, as well as incision due to the presence of several other types of DNA damage in *E. coli*. Seeberg^{7,8} reported that *uvrB*⁺/*C*⁺-complementation activity can be detected from Sephadex gel columns corresponding to ~70,000 MW—this agrees well with our observation that *uvrC* encodes a 68,000-MW protein on SDS gels. Previous attempts

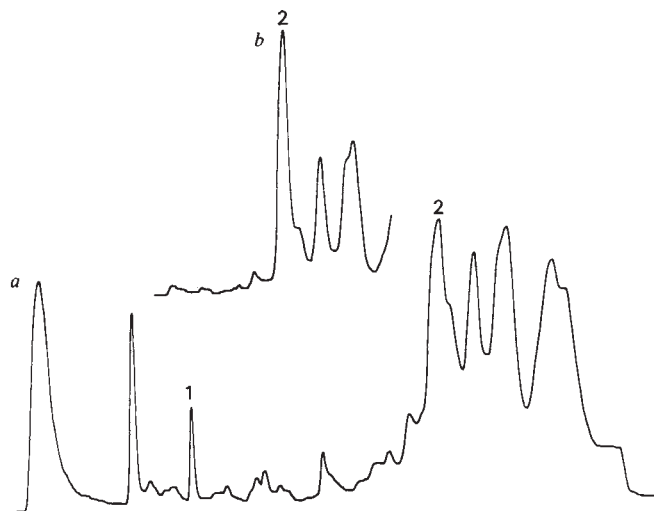


Fig. 3 Densitometric comparison of ^{14}C -labelled *uvrC* and *ssb* protein bands after SDS-polyacrylamide gel electrophoresis of extracts from *uvrC*⁺ *ssb*⁺ *uvrA*⁺ pGHY4610 (ref. 9). The tracing shown in *a* includes the entire gel; the film was exposed for 8 days. The *uvrC* protein band is indicated by 1, and *ssb* by 2. As the *ssb* band is overexposed on this film (*a*), a second film was exposed to this gel for 1.6 days; the result is shown in *b*. The *uvrC* peak (1) in *a* was compared with the *ssb* peak (2) in *b* for this estimate. This comparison was based on the area of each peak, the relative size of *uvrC* and *ssb* polypeptides, and the relative time of exposure for each film. As *ssb* is present in 1,200 copies per cell²² and its molecular weight is 18,500 (ref. 22), these data provide an estimate of ~10 *uvrC* polypeptide chains per log-phase *E. coli* cell, assuming that there is a similar ratio of expression *in vivo* and in maxi-cells for *uvrC* and *ssb*.

to determine the mechanism of dimer-specific endonucleases in *E. coli* have been hampered by the twofold problem of identification of the elements of a 3-gene system (that is, *uvrA*, *uvrB* and *uvrC*) and the presence of very low amounts of UV dimer-specific activity. Our identification of the *uvrC* protein as a product from a multicopy plasmid provides a means to purify *uvrC* protein in sufficient quantities to allow its characterization

as a component of the 3-gene product system required for incision of UV light-irradiated DNA.

We thank Dr Roger L. McMacken for comments during the study and preparation of the manuscript. This work was supported by American Cancer Society grant NP8K, and by National Institutes of General Medical Sciences grant 2 R01 GM22846 to L.G.

Received 6 January; accepted 26 March 1981.

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Positively activated transcription of λ integrase gene initiates with UTP *in vivo*

U. Schmeissner*, D. Court*, K. McKenney† & M. Rosenberg†

*Laboratory of Molecular Biology and †Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

Bacteriophage λ requires two viral gene products for lysogenic development—repressor (*cI*) and integrase (*int*). Although the genes for these two proteins are located in distinct operons, separated by 9,000 base pairs (bp) of the phage genome, their expression is coordinated by the positive regulation of transcription mediated by another phage-encoded protein, *cII* (see ref. 1 for review). We have already identified the promoter signal for the establishment of repressor synthesis (P_E) and demonstrated that *cII* protein is required for the initiation of transcription at this site². Here we determine the precise *in vivo* start site for *int* mRNA and demonstrate that transcription from this site is also *cII*-dependent. This start site defines the *int* promoter P_I . Atypically, transcription from P_I initiates with UTP. Comparison of the nucleotide sequences of the P_I and P_E promoters reveals a striking homology in identical positions between 26 and 39 bp upstream of their respective start sites.

Our objective was to describe the mechanism of transcription in the *xis-int* region of phage λ and to study the effect thereon of *cII* expression (see ref. 2). A 417-bp λ DNA fragment, extending from 280 bp upstream of the *xis* coding sequence to include 150 bp of the *xis* gene as far as the structural *int* gene^{3–5}, was purified and inserted into the plasmid pBR322 (Fig. 1). The DNA of the recombinant plasmid (pUS3) was used to purify RNA made from this region during phage infection by a single-step hybridization procedure (see Fig. 2 legend and ref. 2). RNA was labelled by exposing cells to ³²P-orthophosphate between 10 and 11.5 min after infection—an interval chosen to minimize degradation of the mRNA. This specific RNA (Fig. 2) was characterized by T1 ribonuclease digestion and separation of the resulting oligonucleotides by a two-dimensional fingerprinting technique⁶. The T1 fingerprints were prepared from RNA isolated from cells infected with either λ cII[–] (Fig. 2a) or λ cII⁺ (Fig. 2b). The major (numbered) oligonucleotides were further digested with pancreatic ribonuclease and the products separated by one-dimensional electrophoresis on DEAE paper at pH 3.5 (ref. 6). The results of these analyses allowed us to determine the composition of each T1 oligonucleotide and to position it unambiguously within the known DNA sequence^{3–5} of the 417-bp fragment (Fig. 3).

RNA from λ cII[–] infection yielded equimolar amounts of oligonucleotides spanning the entire fragment (Fig. 3, oligonucleotides 1–9 and 11–17), indicating uniform transcription throughout the region; transcription probably originates at the upstream promoter P_L (Fig. 1). In contrast, for RNA from λ cII⁺ infection, the intensity of oligonucleotides 11–17 was increased some eightfold, whereas the intensity of oligonucleotides 1–9

Fig. 1 Partial genetic map of phage λ . Indicated are the two *cII*-dependent promoters P_E (responsible for the establishment of repressor synthesis) and P_I (responsible for the production of integrase). Other λ promoters (P_L , P_R , P_O , P_M)¹⁰ are also shown, as is the direction of transcription from these sites (wavy arrows). The 417-bp DNA restriction fragment shown below the map was inserted into the plasmid pBR322 by blunt-end ligation. The resultant plasmid (pUS3) was used in the hybridization experiments as described in the text.

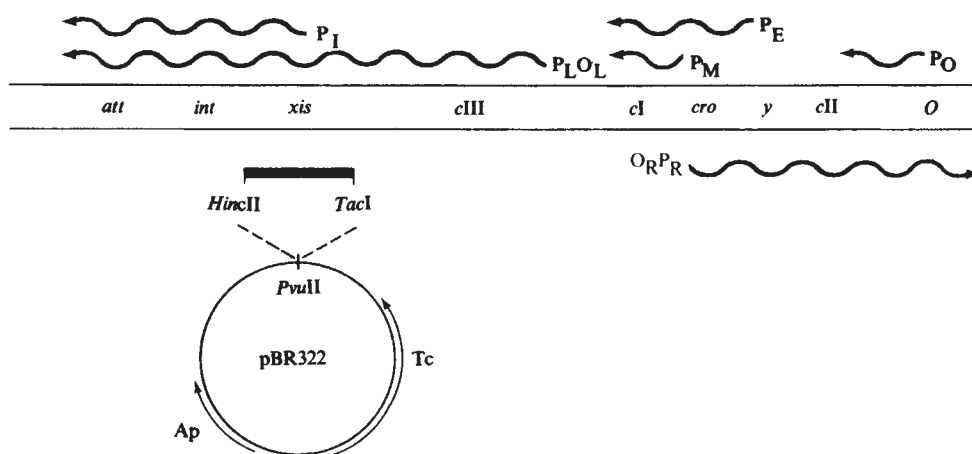


Fig. 2 Two-dimensional fingerprints of ribonuclease T1 oligonucleotides derived from RNA transcribed *in vivo* from the *xis-int* region of phage λ . Fingerprints were prepared from RNA isolated after infection of *Escherichia coli* K12 SA500 (*his*, *rpsL*) with λ CI₁₄II₂₈III₆₁₁ (a) or λ CI₁₄ (b)¹¹. Total RNA was extracted as described by Court *et al.*¹² and hybridized to plasmid pUS3 DNA bound to filters. Hybridizations were carried out at 67 °C for 6 h as described elsewhere². RNA purified by this single-step hybridization procedure was digested with ribonuclease T1 and the products separated by standard fingerprinting techniques. Horizontal dimension, electrophoresis on Cellogel strips at pH 3.5. Vertical dimension, homochromatography on thin-layer plates of DEAE-cellulose using Homomix C. The numbered oligonucleotides in each fingerprint were further analysed by digestion with pancreatic ribonuclease⁶. The results of these analyses are summarized in Fig. 3. In addition, oligonucleotide no. 10 was treated with nuclease P₁ and the resulting mononucleoside-5' phosphates fractionated by two-dimensional chromatography². The results (not shown) indicate that this oligonucleotide contains the triphosphate, identified as pppU. Oligonucleotides 1 to 5 are not shown in Fig. 3; these correlate with the DNA sequence 5' to that shown in Fig. 3 (that is, beyond residue 1,434). Their sequences are: 1, AU₃AU₄AUCUG; 2, AUUA₃UG; 3, CACUCCG; 4, CCAUUCACAG; 5, AACAUCAACG. Oligonucleotide no. 18 has the sequence ACUUA₃UCG and derives from the region immediately 3' to the DNA fragment used for hybridization. The presence of this oligonucleotide probably results from the mild ribonuclease trimming conditions used to preserve the 5' end of the *cII*-dependent transcript during the isolation of the RNA-DNA hybrids.

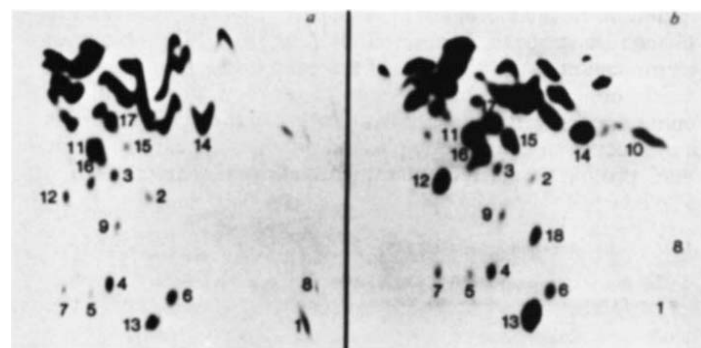


Fig. 3 Nucleotide sequence of the P_I promoter region³⁻⁵. Only the coding strand of the DNA is shown. The orientation of the sequence is inverted relative to the position of λ genes indicated in Fig. 1. The numbering of the nucleotide positions starts in the centre of the λ attachment site⁴. Numbered brackets designate the corresponding oligonucleotides in the RNA fingerprints of Fig. 2. The 5' end of the RNA is indicated by pppU, and the -10 region of the promoter is boxed. Note that the transcriptional start point of the *int* mRNA lies within the *xis* coding region. This is consistent with the finding that the expression of *xis* is not controlled by *cII* (ref. 13). The arrows define a region of dyad symmetry, which is followed by the sequence T₆C. Such a structure is common to sites signalling termination of transcription¹⁴. However, the relative molar yields of oligonucleotides 7, 8 and 9 indicate that the transcription does not terminate at this site during our analyses.

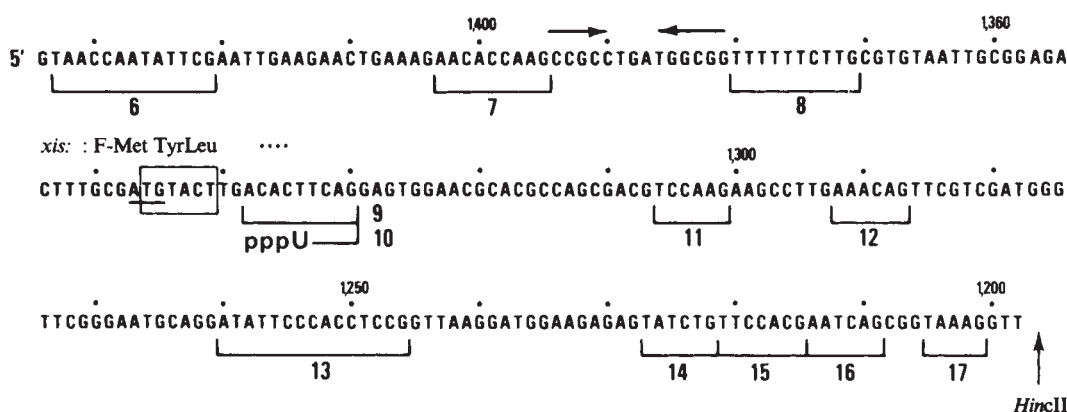
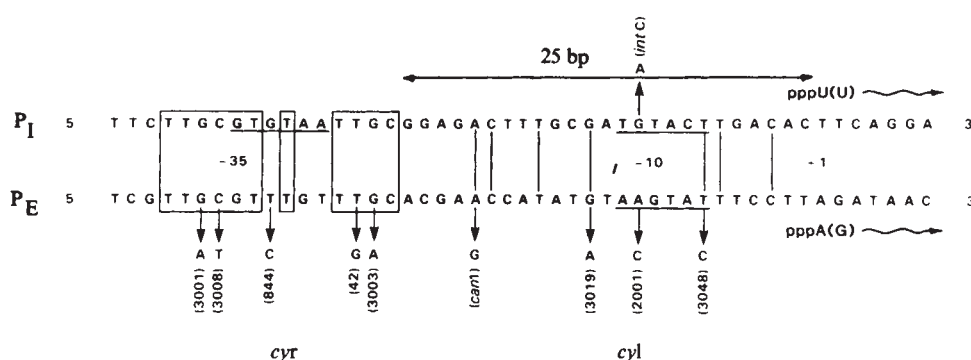


Fig. 4 Comparison of the nucleotide sequence of the P_I and P_E promoters. Only the coding strands of the DNA are shown. The sequences are aligned with respect to the *cII*-dependent transcriptional start sites used *in vivo*. The nucleotides in the -10 region of both promoters and in the -35 region of the P_I promoter, which show some homology with the conserved sequences found in other promoters for *E. coli* RNA polymerase¹⁴, are underlined. The boxed regions indicate 11 of 14 bp which are identical in both promoters. The various *cyr* and *cyl* mutations⁹ which eliminate P_E promoter function are indicated, as in the *can1* mutation¹⁵ which alters the second codon of the *cII* structural gene but has no effect on P_E promoter function. Also shown is the *intC* mutation¹⁶ which occurs in the -10 region of the P_I promoter and results in *cII*-independent, constitutive expression of gene *int*.



was similar to that from λ CI⁻ infection. In addition, a new oligonucleotide (no. 10, Figs 2, 3) was identified as the pentamer pppUUCAG. These results indicate that in the presence of *cII*, a new transcript starts at a site located 183 bp before the translation initiation codon of *int* and 13 bp downstream of the AUG initiation codon of *xis* (at position 1,334 in Fig. 3 as numbered from the centre of the core of the *att* site⁴). This *cII*-dependent RNA accounts for over 80% of the *int* gene transcripts made during the pulse-labelling interval.

Identification of the 5' end of this RNA defines the *cII*-activated P_I promoter.

Note that the P_I transcript initiates with UTP. This is the first *in vivo* demonstration of the use of UTP by RNA polymerase to initiate transcription. Recently, it has been shown⁷ that purified *cII* protein causes RNA polymerase to initiate transcription *in vitro* at the same UTP start. This demonstrates that the positive activation observed at P_I *in vitro* accurately represents the *in vivo* situation.

Hoess *et al.*⁴ and Abraham *et al.*⁵ have determined the base change associated with the mutation *intC* (Fig. 4) that allows *cII*-independent, constitutive expression of *int*. More recent work⁸ indicates that the *intC* mutation also allows RNA polymerase to initiate transcription *in vitro* from the same UTP start as demonstrated here. Our finding confirms the earliest suggestion that *intC* is a promoter-up mutation affecting the -10 region of the P_1 signal^{4,5,8}.

We have compared the nucleotide sequences of P_E and P_I by aligning them with respect to their *in vivo* start sites. Homology in 11 of 14 bp is observed at identical positions in the -35 region of both promoters. This finding further supports the proposal^{2,8,9} that this region of the promoter is involved in the positive activation mechanism, perhaps as a site of interaction with protein *cII*.

We thank J. Abraham and H. Echols for communicating results before publication, R. Steinberg for photographic work and P. Donoghue for typing the manuscript.

Received 23 February; accepted 28 April 1981.

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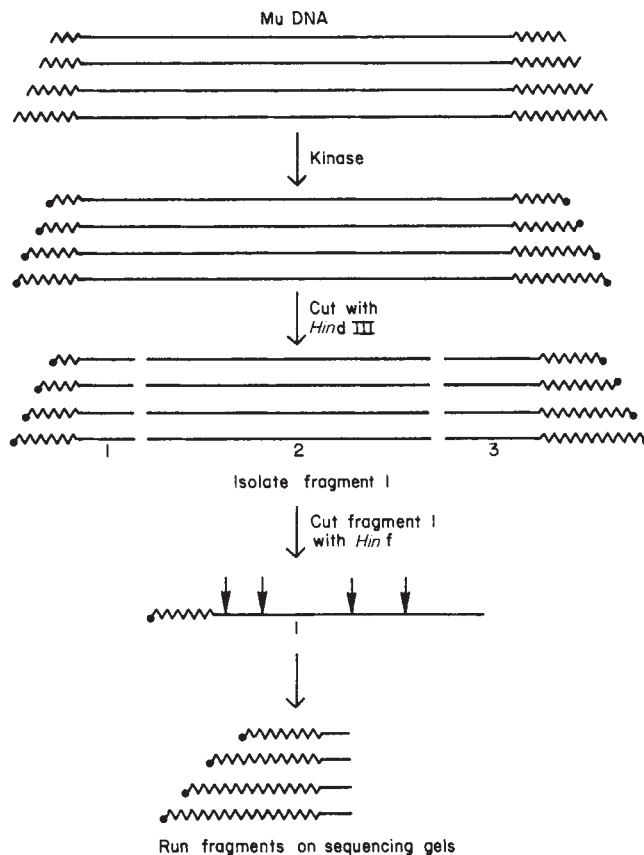


Fig. 1 The experimental design. A single line represents double-stranded Mu DNA. Wavy lines represent host DNA (not drawn to scale). Solid circles represent ^{32}P groups at the 5' ends of the DNA strands.

Heterogeneous host DNA attached to the left end of mature bacteriophage Mu DNA

Marie George & Ahmad I. Bukhari

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

Bacteriophage Mu, a highly efficient transposon during its lytic cycle in *Escherichia coli*, is always associated with the host DNA¹. In mature Mu particles, both ends of Mu are heterogeneous in length and sequence owing to the presence of host DNA²⁻⁶. The right end contains host sequences which vary between 500 and 3,200 base pairs (bp), while the left end heterogeneity was proposed⁷ to correspond to ~50-100 bp. To understand the mechanism by which a small amount of host DNA at the left end is packaged along with Mu DNA, we have now determined the precise lengths of these host sequences. The minimum size of the host sequences attached to the left end of the mature bacteriophage Mu DNA is 56 bp; host sequences longer than 144 bp are rare. The host sequences do not show a continuum from 56 to 144 bp but are rather packaged in discrete blocks. The first such block is 56-61 bp, the second is 67-72 bp and so on. Thus, each block represents fragments covering a 5-6-bp range, with a space of 5 bp between each block. It seems that Mu DNA is being measured in units of helical turns for packaging.

The ends of Mu DNA were labelled with ^{32}P using [γ - ^{32}P]ATP and polynucleotide kinase. Mu DNA was then digested with *Hind*III, which makes two cuts in the DNA, giving rise to three fragments. The 1,000-bp fragment containing the left end of Mu was isolated and cut with *Hinf*, which makes four cuts in the fragment, one of which is 7 bp from the left end of Mu. The digest was then run on a sequencing gel to size the molecules. The experimental design is shown in Fig. 1.

Figure 2 shows the results of the experiment. The smallest fragment obtained is 63 bp. As *Hinf* cuts 7 bp away from the Mu end, the minimum size of the host sequences at the left end must be 56 bp. As the largest fragments are in the range of 150 bp, the maximum size of the host sequences is ~144 bp. However, smaller fragments are much more frequent, and only a small minority of the fragments are larger than 100 bp, while fragments larger than 150 bp are extremely rare. The most striking feature of the size of the host sequences is that they are not present as a continuum from 56 to 144 bp, but rather appear in discrete blocks. Moreover, there is a regular pattern in which these blocks appear: the first block is from 63 to 68 bp; a gap of 5 bp is then seen, after which there is another block from 74 to 79. There are 10 such blocks, each one separated from the next by a 5-bp space, and each containing fragments covering a 6-bp range. This means that DNA cutting begins with a regular spacing of 5 bp but cutting can cover any of the next 5-6 bp. Analysis of the end nucleotides, by labelling of the end and analysis of the 5' nucleotide, showed that all four bases are present in equal amounts at both the left and right ends.

Two general modes of packaging of DNA into viruses have been recognized^{8,9}. One is site-specific packaging with some size constraints, such as the recognition of the *ter* sites of λ for packaging, and the other is the headful packaging, in which the size of DNA seems to have the predominant role. Packaging by a

headful mechanism was first proposed by Streisinger *et al.*¹⁰ for phage T4, and has been shown to be a common mode of DNA packaging^{5,11,12}. However, the molecular mechanism of headful packaging is unclear. In context with Mu packaging, A.I.B. *et al.*³ discussed two alternative ways in which a small amount of host DNA can be packaged at the left end. In one mechanism, a specific site at the left end is recognized by a DNA cutting protein, the DNA is then cut to the left of the site randomly, and the cleaved DNA condensed into the heads being assembled. In

the second mechanism, a packaging protein recognizes a specific sequence at the left end, the DNA is then folded and perhaps pushed into the heads being assembled, after which the DNA is cut at the left and right end.

It has been observed that the phage tail is attached at the right end of Mu^{13,14}; presumably, the right end is the last to be packaged, and the first to be ejected, suggesting that the left and right ends are being cut in different time and space. However, the minimum size of the host DNA at the left end is ~56 bp. It is difficult to reconcile this observation with the first mechanism in which the first event is cutting of the left end by an enzyme behaving like a type I restriction enzyme. The enzyme would have to recognize a site on the left end but start cutting only after 56 bp. As a working hypothesis, we favour the idea that the DNA is rolled or packaged first, in such a way that the first 56 host base pairs at the left end are not available for cutting. This could either mean that the left end is condensed first, and cut, after which the rest of the DNA is packaged in, or that the whole genome is packaged, and then the left and right end cuts are made.

How can we explain the regular spacing of the cuts? Note that the distance from the beginning of one block of 6 bp to the beginning of the second block is ~11 bp, which is about one turn of the double helix. It seems that the DNA is being measured for packaging, in units of helical turns. This measurement could be brought about by proteins that bind at each turn of the helix. The proteins perhaps cover one half of the turn so that only the other half is available for cleavage.

This work was supported by grants from the NIH (GM23566) and NSF (PCM7826710).

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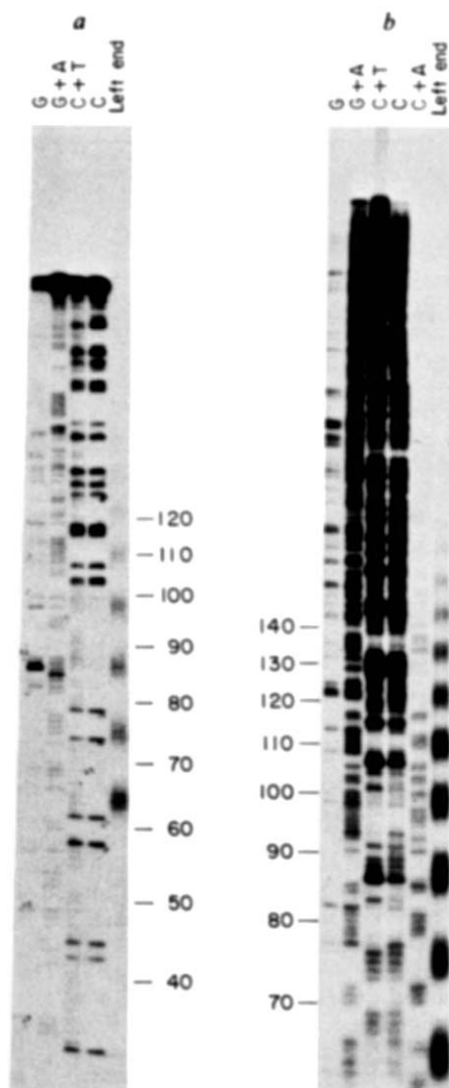


Fig. 2 Determination of the lengths of the left-end host sequences by electrophoresis in sequencing gels. Bacteriophage Mu particles were purified by a caesium chloride density gradient centrifugation. The DNA was extracted with phenol. To label the ends, the 5' phosphates were first removed by treating the DNA with alkaline phosphatase¹⁵. The ends were labelled using [γ -³²P]ATP (>2,000 Ci mmol⁻¹) and polynucleotide kinase. The DNA was then cut with *Hind*III and the digest run on a 6% 1:40 acrylamide gel. The 1,000-bp left-end fragment was isolated¹⁵ and cut with *Hinf*I. The digest (left end) was run on 8% polyacrylamide gels containing 7 M urea, together with DNA samples that were being sequenced; *a* and *b* are autoradiograms of 8% sequencing gels run for different times. The xylene cyanol dye marker was three-quarters of the way down (*a*) or off (*b*) the gel. Portions of double-stranded DNA, labelled with ³²P at one 5' end, were partially cleaved at guanines (G), guanines and adenines (G+A), cytosines and thymines (C+T), cytosines (C), and cytosines and adenines (C+A), respectively, using the method of Maxam and Gilbert¹⁵. The numbers indicate the size of the fragments as seen on the sequencing gels.

Errata

In the article 'IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts' by P. J. Gearhart *et al.*, *Nature* **291**, 29–34 (1981), Figures 1 and 2 were transposed.

In the letter 'Somatic and behavioural postnatal effects of fetal injections of nerve growth factor antibodies in the rat' by L. Aloe *et al.*, *Nature* **291**, 413 (1981), the received date was given as 7 November 1980. The paper was originally submitted on 7 November 1979; the date of receipt of the revised manuscript was 17 November 1980.

The cover caption for issue no. 5813 (28 May–4 June) of *Nature* was incorrect. The correct version from the authors is 'Homozygous (pigmented and unpigmented) progeny from heat-shocked eggs of heterozygous mothers'.

MATTERS ARISING

Sensory cues invalidate remote viewing experiments

TART, Puthoff and Targ¹ have recently responded to our critical analysis^{2,3} of their evidence in favour of extrasensory remote viewing ability by reporting a re-judging of the original transcripts with all sensory cues removed. This yielded a high correlation between Price's descriptions and the target information. Furthermore, Tart *et al.* claim that the Marks-Kammann cueing explanation of remote viewing does not apply in principle to any of the replication experiments carried out after the series with Price.

The validity of the re-judging exercise for the Price series can be disputed on two counts. First, it should be noted that the editing of transcripts was carried out by one of the investigators (Tart). As Tart was himself aware of the correct target-transcript pairings, this could have led to biasing. Second, it is not permissible to include material for re-judging which has already been published or which may be available in some other form. A so-called 'blind' judge may have some memory of previously seen target-transcript matchings or have access to the published material. Only five of the series of nine targets and transcripts for which there is no normal or available method of matching except perceived similarity, could validly be used in re-judging. The remaining four transcripts should have been excluded, as in the unsuccessful re-judging exercise reported by us².

A much more serious problem with the response of Tart *et al.*¹ to our report is their claim that sensory cues were not present in later experiments. Unfortunately, I have been unable to obtain a complete set of transcripts from SRI investigators despite frequent requests. However, in June 1977, Dr Arthur Hastings, who was a consultant to the SRI investigations responsible for judging experimental transcripts, allowed me to see six transcripts from the series with the subject H. Hamid, and I was far from satisfied with them as they contained sensory cues. A listing of targets in Hastings' possession correlating 0.83 ($P < 0.01$) with the order of target usage, together with transcript cues, would have provided an artefactual basis for correct target-transcript matchings.

Although Tart *et al.*¹ conclude that SRI replication studies confirm the remote viewing hypothesis, serious methodological flaws throughout the experiments prohibit any such conclusion. The Targ-Puthoff researches conform to a long history in parapsychology of methodological flaws and mistaken conclusions. Unless proper controls and methods are used by impartial observers, the search for

scientific proof of paranormal and spiritual beliefs remains a futile enterprise.

DAVID MARKS

Department of Psychology,
University of Otago,
Dunedin, New Zealand

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Micrograzers may affect macroalgal density

THE EFFECT of density on plant growth and mortality has been recently discussed by Schiel and Choat¹. Studying two species of large brown seaweed, *Ecklonia radiata* and *Sargassum sinclairii*, they found that high density had a positive effect, in contrast to data for terrestrial plants. They concluded that density affects marine and terrestrial plants differently, and suggested that these differences were due to protection from wave shock and the difference in plant-arthropod associations between terrestrial and marine environments. Uninjured brown algal thalli contained up to 1,700 copepods, amphipods and isopods.

Terrestrial plant density has a limiting effect on nutrient and water supplies to the individual plant that would not be expected for algal density, but we believe that the data for *Sargassum* and *Ecklonia* communities¹ are exceptional and subject to reinterpretation. Specifically, conclusions on the general effect of density on marine plants should await data from algal typical of less exposed communities.

Our work on amphipod grazing in the field and in the Smithsonian Institution's coral reef microcosm demonstrates that coarser algae (for example, *Hypnea*) are protected from amphipod grazing by their size, but most filamentous species are heavily grazed². By eliminating epiphytes on coarser algae, amphipods increase growth rates of macroalgae such as *Hypnea* by as much as 300%. In subtidal areas, amphipod densities are kept low by fish predation³, but in situations where this is reduced by factors such as turbulence, micrograzer herbivory may be particularly important. Such high energy areas are characteristic habitats for brown algal macrophytes. Up to a point, densely growing *Ecklonia* and *Sargassum* plants would shelter more amphipods from predation. This would contribute to a positive effect of high density on these seaweeds, while being equivalent to a locust attack in its effect on many other algal species.

Schiel & Choat¹ suggest that the study of marine plant-arthropod relationships could have important implications for mariculture. In fact, our work on

amphipod grazing implies that maintenance of some amphipod species in mariculture facilities could increase yields significantly.

SUSAN H. BRAWLEY
WALTER H. ADEY

Marine Systems Laboratory, W-310,
Department of Paleobiology,
Smithsonian Institution,
Washington DC 20560, USA

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SCHIEL AND CHOAT REPLY—Our recent paper¹ reported two of our findings for large marine algae: (1) individual plants in dense, monospecific stands tend to be larger compared with plants of the same age in sparser stands, and (2) the number of crustacea in algal fronds may be quite high with no apparent adverse effect on algal plants. We contrasted this to terrestrial plant systems, where the opposite seems to apply in both cases. These observations were put forward as hypotheses worthy of further testing.

An experimental demonstration of a link between fish predators, frond-dwelling crustacea and the occurrence of epiphytes on large brown algal plants has proved largely intractable in field situations. Data on the comparisons of arthropod loads between densities and exposures of plants, the fish effect on arthropod loads, and the effects of arthropods on host plants are either lacking altogether or equivocal, particularly over large areas generally. Brawley and Adey² have provided evidence in their coral reef microcosm that such a link exists, and that high numbers of crustacea may be of benefit to larger algae by reducing epiphytism. Their results do not negate our hypotheses. Our argument admits the likelihood of a beneficial effect on plants, or no effect at all, due to crustacean loads. However, before a general case is made more data are required concerning: (1) the general effects of density on growth, survival and size of marine plants; (2) the effects of crustacea on marine plants, and (3) the effects of fish predators on crustacea. Brawley and Adey have provided some information. We welcome further testing of the hypotheses we have put forward.

DAVID R. SCHIEL
J. H. CHOAT

Department of Zoology and
Leigh Marine Research Laboratory,
University of Auckland, Leigh,
New Zealand

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The glass bead game

THE gap between theoretical and applied ecology seems to be widening. Indeed it is difficult to escape the worrying conclusion that some theoreticians are playing a version of Hermann Hesse's *Das Glas-perlen Spiel*¹ or that they have little feeling for or understanding of biological problems. A particularly good example is the recent article by Gurney *et al.*² which attempts to provide a theoretical model to explain the oscillatory behaviour of laboratory populations of blowflies.

After reaching the esoteric conclusion that the blowfly cycles are "self-sustaining limit cycles" rather than "driven quasi-cycles", the authors proceed to use their model to explain the 'double-humped' nature of the cycles in terms of minimum population size ($N_{\min}$) in relation to the size at which the population achieves maximum reproductive success (N_0). The mathematics are correct, but in their zeal, the authors fail to notice Nicholson's own explanation of the 'double-hump' phenomenon (see Fig. 3 legend in ref. 3). He says "the lack of a clear inverse relation between the various low adult densities and the number of eggs produced is due to the fact that adults are mostly senile as the adult minima are approached, near the minima many are newly emerged and incapable of laying eggs, and subsequently highly fertile young individuals dominate". In other words, the fact that breeding occurred in 'quasi-discrete' generations is probably almost entirely a consequence of age-specific variation in the reproductive performance of the adult blowflies. As Gurney *et al.* ignore this variation their model must be seen as artefactual and spurious—a product of the 'game'.

Of less importance, but still worrying, is the promotion of "a satisfying qualitative fit" in the conclusions to "good quantitative agreement" in the abstract, and the failure to refer to similar published work^{4,5}. In contrast, Readshaw and Cuff⁶ have published a biologically realistic model of Nicholson's results which includes readily identifiable parameters. An age-specific version of the model would undoubtedly simulate the 'double-hump' but the data are not yet available.

J. L. READSHAW

CSIRO, Division of Entomology,
PO Box 1700,
Canberra City, ACT 2601,
Australia

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GURNEY *ET AL.* REPLY—Readshaw raises three scientific objections to the model described in our recent article¹. We shall deal with these in turn.

The question of the nature of the mechanism responsible for the observed cycles is very far from 'esoteric'. Any moderately repetitive fine structure exhibited by a limit-cycle type of fluctuation carries readily extractable dynamic information, whereas the fine structure of a driven quasi-cycle is mainly 'noise' which only serves to obscure our view of the underlying population dynamic. Thus our judgement that detailed investigation of the fine structure of the cycles observed by Nicholson is a worthwhile exercise hinges on our unambiguous demonstration that they are limit cycles.

It is clear from our work that if average future recruitment bears any kind of humped relationship to current adult population then cycles which have minima well below the population size at which maximum overall reproductive success is

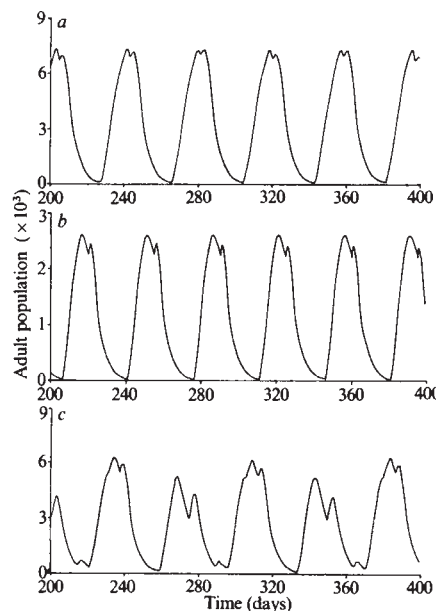


Fig. 1 Numerical solutions of Readshaw and Cuff's model equation¹: $n(t+1) = 0.8n(t) + R(n(t-\tau))$. *a*, Adult food-limited case: $R = 10n$ when $n < 171$; $R = 1,795 - 0.503n$ when $171 < n < 3,569$; $R = 0$ when $n > 3,569$. $\tau = 15$ days. *b*, Larval food-limited case: $R = 10n(1 - \exp\{0.154 - 109.9/n\})$ when $n < 714$; $R = 0$ when $n > 714$. $\tau = 13$ days. *c*, Adult food-limited case with modified parameters: $R = 2.49n$ when $n < 600$; $R = 1,795 - 0.503n$ when $600 < n < 3,569$; $R = 0$ when $n > 3,569$. $\tau = 15$ days.

achieved, must be accompanied by a 'discrete generation' pattern of breeding activity. A clear implication of Nicholson's^{2,3} batch culture results displayed in Readshaw and Cuff's paper⁴ is that just such a relationship exists for *Lucilia cuprina*, and there is thus no shred of evidence for their *ex cathedra* statement that the double-humped egg-laying rate curves observed by Nicholson are entirely the product of the age structure-dependent fecundity changes noted in Fig. 3 legend of ref. 3. However, such effects do provide a very plausible explanation of the observation that in four out of seven cycles

shown the second peak of the double hump is considerably higher than the first.

The final objection of Readshaw is the claim that the fine structure predicted by our model must be 'spurious and artefactual' because their 'biologically reasonable' model predicts a limit cycle with no fine structure. This claim has no sound basis. Their model is effectively identical to ours except in the details of the functional form chosen for the recruitment rate function. In both experimental regimes considered the form chosen has a single hump with a maximum at a population size (N_0) comfortably in excess of the observed minimum population and thus there seems every reason to suppose that careful numerical analysis will reveal that their model predicts population cycles with a fine structure very similar to that shown in Fig. 6 of our paper¹. Figure 1*a, b* shows that this is indeed the case. Furthermore, in the adult food-limited case (Fig. 1*a*) note that there is no direct experimental evidence for the value of N_0 implied by the parameters chosen by Readshaw and Cuff (171) and indeed that this value is considerably below the value of 600 that may be deduced from Nicholson's data (see Fig. 3 of ref. 3). If we abandon the attempt to force Readshaw and Cuff's piecewise linear approximation to the recruitment function to fit the behaviour of the population as $N \rightarrow 0$ and instead place the maximum of the curve somewhere near the correct value (Fig. 1*c*), then the structure predicted by their model becomes very strong indeed.

We conclude that the very simple mechanism proposed in our original article captures much of the spirit of the population dynamics underlying Nicholson's blowfly cycles and is thus a contribution to narrowing (rather than widening) the gap between theoretical and applied ecology.

W. S. C. GURNEY
S. B. BLYTHE
R. M. NISBET

Department of Applied Physics,
University of Strathclyde,
George Street, Glasgow G4 0NG, UK

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Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

BOOK REVIEWS

Unravelling the threads of cosmic history

James N. Fry, Craig Hogan and David N. Schramm

CLASSICAL physical cosmology, as exemplified by texts such as Weinberg's *Gravitation and Cosmology* (Wiley, 1972), deals with an idealized "smooth" Universe taken as a whole, its overall dynamics and mean composition. General relativity describes the universal expansion in an average sense, assuming that the material content is very nearly uniform. All standard calculations, such as the synthesis of primordial helium, rely on this background. Astronomers, on the other hand, have always studied discrete concentrations of matter, the galaxies for example, but until recently many regarded cosmology as a "study of the search for two numbers" — the expansion rate and the deceleration parameter of the smoothed substratum.

However, the detailed study of the distribution of galaxies, their clustering and their peculiar (non-Hubble) motions, forms a fascinating subject in its own right, which offers a great variety of problems, dynamical, statistical and observational, for both the astronomer and the physicist. Here there exists the possibility of unravelling some of the threads of cosmic history. Jim Peebles has written the first textbook to deal almost exclusively with what might more appropriately be described as the "in-between-scale structure of the Universe" — bigger than galaxies, but much smaller than the observable Universe.

On the very largest scales, above about 10 Mpc (30 million light years), the Universe is indeed nearly uniform, as is indicated by such diverse measures as the isotropic distribution of galaxies, faint radio sources, the X-ray background radiation, and especially the microwave background radiation, which of all these comes from farthest away and reflects the oldest parts of the Universe we can reach — its last interaction with matter probably occurred when the Universe was about 1,000 times smaller than it is now. On smaller scales, less than 10 Mpc, the distribution of galaxies displays a rich structure, illustrated in the frontispiece of Peebles's book and also in a beautiful poster ("One Million Galaxies" published by Peebles and Stewart Brand of *CoEvolution Quarterly*). The structure can be quantified by statistical measures such as the galaxy correlation functions, and it is consistent with the model that galaxies are distributed in a sort of self-similar nested

The Large-Scale Structure of the Universe. By P. J. E. Peebles. Pp.416. ISBN hbk 0-691-08239-1; ISBN pbk 0-691-08240-5. (Princeton University Press: 1981.) Hbk \$30, £18.10; pbk \$9.95, £6.20.

hierarchy — galaxies are bound in groups which orbit each other in clusters and so on, up to scales of 3–10 Mpc where the largest aggregations blend smoothly into the uniform background.

From its gravitational effects on luminous galaxies, we now know that most of the mass of the Universe is dark. We cannot tell from these dynamical studies what the mass is made of, although there are some constraints from big bang nucleosynthesis on the total baryonic component. Depending in part on how the total cosmological mass density compares with these constraints on baryon density, the dark matter might be anything from swarms of invisible particles, such as neutrinos, to clusters of very dim, low mass stars, to black holes as large as a million solar masses each. Nevertheless, if the distribution of mass is similar to the visible galaxy distribution then natural mechanisms exist for creating a hierarchical structure. (One must be careful here, since there is some evidence that the dark matter may have a different spatial distribution from the light-emitting matter.) In one picture, as the Universe expands and cools, galaxies (with their dark counterparts) condense out of the primordial matter. Neighbouring galaxies gravitationally pull each other out of the expansion and start orbiting each other, forming groups; neighbouring groups approach each other, and so on. Opposed to this is an alternative view — that the first scales to condense are very large, about 10 Mpc, and that flattened gas clouds of this size ("pancakes") fragment into smaller ones, which break up into still smaller ones, and so on, finally reaching galaxy sizes. Both frameworks seem viable and are intuitively satisfying. It remains a fascinating enigma exactly how a nearly uniform expanding Universe produces the structure we see. This area of cosmology is at an interesting stage of development because, although the expanding Universe option is widely used, various detailed models (to explain the same observations) differ in almost all essential aspects. Fortunately, the differing predictions are on the verge of being tested, primarily by

observations of anisotropy in the microwave background, our best direct probe of the epoch just prior to galaxy formation.

Peebles's book supplements the standard textbook version of cosmology with the results of these structural studies. If you are active in research dealing with cosmological structure, you must have a copy, for it is the most complete source for finding out what has been done. However, for those with only casual interest, the book is much less effective. The approach is that of a pioneer in the field, recollecting calculations and lines of thought from over the years, and it is thus extremely valuable as a reference; but this does not make for ideal pedagogy. Except for the historical introduction, there are few pointers in the 400 pages toward which material is likely to remain important; current fashion and fundamentals are treated with almost equal weight. If you know specifically what you want to know, you can find it in this book — unlike Peebles's previous book, *Physical Cosmology* (Princeton University Press, 1973), it does have an index; every standard calculation is here (there are four frameworks for deriving the linear growth equations) and there is an extraordinarily complete list of references. But if you are an outsider and want to get a picture of what is going on, what is likely to remain relevant and what may be tossed out in the next ten years, you will probably be lost.

Peebles does not attempt to conceal his biases, and exercises the author's right to present a convincing case — with a fair assessment of the uncertainties. He emphasizes the idea of a "well-regulated", quiescent initial state replacing the formerly popular ideas of primordial chaos, turbulence, magnetic fields; the hierarchical clustering picture emerges as the one simple physical process which can make solid predictions about the galaxy distribution; the Universe contains a lot of unseen matter, possibly enough to make the daring statement $\Omega = 1$.

Despite the possible shortcomings, there is no question that the field has needed this book. It will undoubtedly become a standard graduate extragalactic astronomy textbook. Here is a collection of useful calculations which were not previously available in any one place (although some of the book overlaps with S. M. Fall's excellent review on correlation functions (*Rev. Mod. Phys.* 51, 21; 1979). Even

though there is no complete universal model, there is a great deal of standard lore — correlation functions, newtonian statistical dynamics, relativistic perturbation theory, scaling laws and so on. Peebles avoids the fundamental uncertainties by focusing on manageable physical problems

which can be solved to yield precise answers to specific questions. That is perhaps the best that anyone could do given our present state of ignorance. □

The authors are at the Astronomy and Astrophysics Center of the University of Chicago.

Spectroscopic sound

M. J. Adams

Photoacoustics and Photoacoustic Spectroscopy. Chemical Analysis, Vol. 57. By Allan Rosencwaig. Pp.324. ISBN 0-471-04495-4. (Wiley: 1981.) £19, \$43.75.

The photoacoustic effect (prior to 1977 more commonly termed the optoacoustic effect) provides the basis for a calorimetric method of measuring the absorption of electromagnetic radiation by a sample under study. The phenomenon may be applied to many spectroscopic and non-spectroscopic studies involving the interaction of radiation with matter. Dr Allan Rosencwaig, internationally recognized as a pioneer in the modern development of photoacoustic techniques, has attempted to bring together in this book a comprehensive review of the theory and current applications of methods employing the effect.

In the first two chapters the author presents a brief summary of the phenomenon and describes its study up to the last decade. Until quite recently the majority of applications of photoacoustic spectroscopy (PAS) have been limited to the study and analysis of gaseous systems, and six chapters (approximately a quarter of the book) are given over to this work. The theory and more common experimental arrangements for gaseous PAS are discussed and a chapter is devoted to a review and evaluation of suitable radiation sources for PAS. Applications of PAS to the study of gases, including multi-component analysis, de-excitation studies, high resolution spectroscopy, photolysis and non-linear effects, are examined.

Much of this first part of the book is already available in many excellent reviews and serves here only as a background and introduction to the remainder of the text which covers the application of photoacoustic studies to the examination of condensed-phase media. It is in the contents of these 15 chapters that the author's own contributions are predominant and which provide the book with its special interest. A chapter is devoted to the general theory of the photoacoustic effect employing gas-microphone systems and piezoelectric transducers, and another, of five pages, provides a simplified theoretical account for those who might find the more rigorous treatments, derived in previous sections, demanding. Experimental systems and apparatus for the examination of solid and liquid samples are reviewed and a chapter is reserved for the more novel spectroscopic studies, including dichroism and Fourier transform techniques. The areas of condensed-phase sample PAS reviewed comprise chemical studies, surface studies and applications in biology and medicine. De-excitation phenomena in solid and liquid media, including fluorescence

Rabbie Burns and the art of genetics

Tony Searle

Genetics and Probability in Animal Breeding Experiments. By Earl L. Green. Pp.271. ISBN 0-333-27243-9/0-19-520159-0. (Macmillan Press, London/Oxford University Press, New York: 1981.) £20, \$36.95.

It is well known that even "the best laid schemes o' mice an' men gang aft a-gley", so the need for this book is obvious. It is meant to minimize the "a-gley factor" where the two species undertake joint genetic enterprises, and it should fulfil its purpose if read as conscientiously as the lectures engendering it were doubtless listened to. They were given by Earl Green when Director of the Jackson Laboratory, to each year's new crop of staff members and postdoctoral fellows. No one who is familiar with the high standards of genetic

research and animal breeding at that laboratory can doubt their effectiveness.

Amidst all the marvels of somatic cell hybridization, probes, DNA sequencing and the like, the more humdrum procedures of constructing and maintaining inbred strains for particular purposes, introducing mutant genes into them, studying newly arisen mutants and finding where they belong in the genome must go on. In the past, the necessary expertise has had to be acquired piecemeal and often belatedly; now it is all gathered together in one volume. I could have done with such a vade-mecum 30 years ago, but it (and I) would have been slimmer then. Thus, there is now full coverage of how to produce and use recombinant inbred strains, which have proved so valuable in recent years, and of modern methods of linkage analysis. In fact, the chapters "Linkage, Recombination and Mapping" and "Mating Systems", with associated appendices (for example on maximum likelihood methods) take up nearly half the book.

J.B.S. Haldane used to punctuate his genetics lectures with "Let me give you an example" and Earl Green uses the same idea liberally in this book. Matings of mice with pale ears and dilute coats illustrate probabilities, tests of significance, symbolism, gametic output and so on, other matings illustrate linkage and still others demonstrate the use of Finney's scores for linkage and analysis. These help a lot in what would otherwise be a harder slog. All the examples are based on mice but of course the book as a whole should be a boon to all mammals in search of a genome. The mouse is far ahead of the rest at present, but with modern mutagenic methods the gap could be narrowed quite rapidly.

Among the ten appendices are the complete rules of nomenclature for mouse genes and inbred strains, as well as the author's system of record-keeping and a recommended mouse-room layout. My only regret is that there is so little on how to deal with translocations and other chromosome anomalies. There are a lot of them about nowadays and, with some types of cross, they can play havoc with Mendelian genetics. □

A.G. Searle is in the Genetics Section at the Medical Research Council's Radiobiology Unit, Harwell.

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studies and quantum efficiency measurements, are discussed in detail. There is also a useful account of thermal processes in samples and sample depth profiling, and thickness measurements using the photoacoustic effect are considered. The final two chapters of the book are concerned with more specialized instrumental topics: photoacoustic spectroscopy at low temperatures and photoacoustic microscopy.

During the past decade studies involving the photoacoustic effect have increased dramatically and the range of instrumental methods and applications is now widespread. In producing this book, the first of its kind to deal exclusively with photoacoustics, Dr Rosencwaig has succeeded in including a discussion of all the major topics currently being investigated employing the techniques. With its wide-ranging coverage, the text will be of interest not only to those actively engaged in photoacoustic research but also to spectroscopists seeking a detailed introduction to the technique and its possible use in their own field. □

M.J. Adams is in the Department of Spectrochemistry at the Macaulay Institute for Soil Research, and is a past SRC Research Fellow in photoacoustic spectroscopy.

Earliest technology

J.A.J. Gowlett

Premiers Outils Taillés D'Afrique. By Hélène Roche. Pp.263. ISBN 2-901161-146. (Société d'Ethnographie, Paris: 1981.) 80F. Available from C. Klincksieck, 11 rue de Lille, Paris 7.

"FEEL good" wrote Johanson in his diary just before discovering the early hominid remains of "Lucy". It was good too that Hélène Roche, a French co-worker in the same Afar region of Ethiopia, succeeded in finding the earliest stone tools yet known (c. 2½ million years old), which now gain mention in a book of wider scope. Her book, though, is not so much the popular introduction which the title might imply, but rather a painstaking exercise in the study of early stone technology. English texts on early man abound, but a book in French is rarer, and deserves to find an audience. Central questions are approached thoughtfully: "What is the significance of the acquisition of tools in the process of hominization?" is an issue too often swept aside by currently favoured theories which stress social factors, such as food sharing, in human evolution. Here, in contrast, the flaked stone tools, which preserve sequences of individual decisions from the distant past, are seen as an important aspect in early man's progress, encouraging technical awareness, aptitude and skill.

The heart of the book is a statistically

presented examination of the manufacturing routines employed in fashioning "worked pebbles". It seems a pity that some key sites, including those at Afar, are treated only in the useful introductory chapters, and that the main study hangs on just two samples from Olduvai and two from insecurely dated sites in Morocco – but then, nobody has access to material from all the early sites.

There are some debatable points of terminology or fact (*Australopithecus africanus* has not been found at Baringo), but Roche is surely right to criticize prehistorians for continuing to use terms like "chopper" even though we now intend no functional implication.

Roche concludes that simple tools were fashioned by using only certain preferred flaking sequences, amongst a range of possible routines, thus showing a consistent human desire to "get out" a worked edge from the raw material. But could they have been made differently? Some workers will argue that a worked pebble can only end up in certain forms. Nevertheless, Roche makes perceptive points about early human conceptual abilities, and her book offers much food for thought. □

J. A. J. Gowlett is Senior Archaeologist of the Radiocarbon Accelerator Unit, Research Laboratory for Archaeology and the History of Art, Oxford.

Notes on the birth of experimental psychology

O.L. Zangwill

An Education in Psychology: James McKeen Cattell's Journal and Letters from Germany and England, 1880–1888. Edited by M.M. Sokal. Pp.372. ISBN0-262-19185-7. (MIT Press: 1981.) \$30, £18.60.

WILHELM Wundt (1832–1920), an unsuccessful physiologist who held the Chair of Philosophy at the University of Leipzig for many years, is remembered as the founder of the first laboratory explicitly dedicated to the pursuit of experimental psychology. It opened in 1879 and soon began to attract graduate students from all over the world – and in particular from the United States – drawn it would seem less by the profundities of Wundt's thought than by the promise of his laboratory. Accordingly, it was to Leipzig that a 20-year-old American graduate, James McKeen Cattell, the son of a Presbyterian Minister who was also Principal of the College from which his son had graduated, directed his footsteps.

Cattell studied under Wundt for two years. He then returned to America and for a year held a teaching post at Johns Hopkins University, which however he relinquished in order to return to Leipzig and to become, largely at his own initiative, Wundt's assistant. Their work at this time was largely concerned with the measurement of reaction times, then a very popular preoccupation of psychologists and which has in recent decades regained much interest as a result of contemporary concepts of human information processing. Cattell's work was published in both German and British journals (including *Mind* and *Brain*). He obtained his doctorate at Leipzig in 1886 and spent the greater part of the next two years at St John's College, Cambridge, which has a long-standing reputation as an institution sympathetic to experimental psychology.

Cambridge had at that time no Department of Experimental Psychology and a proposal set in train by James Ward in 1877

that a psychophysics laboratory be instituted was vetoed as disrespectful to the dignity of mind. While unable to proceed further with his experiments on reaction time and associative processes, Cattell's journal and letters report exciting discussions with philosophers such as Ward and Henry Sidgwick, neurologists such as Ferrier and Hughlings Jackson and, most significant of all, the polymath Sir Francis Galton. Indeed in his year or two at Cambridge Cattell acquired from Galton a major interest in the measurement of individual differences in mental capacity which owed nothing to his years with Wundt and which did much to shape his work at Columbia in the earlier decades of this century.

This selection from Cattell's journal and letters, mostly to his parents, together with some of their replies, provides an interesting picture of the impact of Europe on a sensitive and intelligent young American psychologist in the penultimate decade of the last century. While some of his judgements, particularly on matters aesthetic, are naive in the extreme, others reflect an intellectual dedication and whole-hearted devotion to science which might well have led to much more important original research had the young author decided to act on his original plan to remain permanently in Cambridge. (As it was, Cattell's later career was largely that of an organizer of research and an academic administrator, and he cannot be rated as in the top flight of American psychologists of his period.) The material has been assembled and edited with scholarship and care, though it is perhaps a pity that many passages labelled as "gossip" have had to be excluded. If one's interest lies in the man rather than in his work, which appears to be the case here, his gossip may provide much more interesting clues to his character than the details of his work, his travels and his social encounters with the distinguished.

None the less, some interesting items of social history escape the editor's scissors. For example, it is evident that self-experiments with a whole variety of drugs, including caffeine, ether, hashish, laudanum and morphine, were regarded as perfectly in order even in the family of a Presbyterian College President. Such "experiments" appear to have been regarded as healthy explorations of the

range of consciousness and as carrying no social or medical stigma whatsoever. It would be interesting to trace the reasons why such widely disseminated personal idiosyncracies apparently failed to produce anything remotely approaching the student drug culture of today. □

O. L. Zangwill is Professor of Experimental Psychology at the University of Cambridge.

Transformations in magma physics

M.J. O'Hara

Physics of Magmatic Processes. Edited by R.B. Hargraves. Pp.585. ISBN hbk 0-691-08259-6; ISBN pbk 0-691-08261-8. (Princeton University Press: 1980.) Hbk \$40, £24; pbk \$15, £9.30.

THIS companion volume to *Evolution of the Igneous Rocks; Fiftieth Anniversary Perspectives* (Princeton, 1979) emerges from a conference commemorating the fiftieth anniversary of Bowen's great publication. Written by an impressive list of experts, it contains 11 chapters on a range of aspects of the physics, thermochemistry and trace element chemistry of magmas.

In the opening contribution, P.C. Hess discusses the structure of silicate liquids and indicates the importance of its influence on liquid immiscibility and distribution coefficients for trace elements. His account, though useful, would have been more valuable with the inclusion of the instructive saga of the variations of nickel distribution between olivine and liquid, and if the implications of changes in liquid structure with pressure (and with time in an experiment) for measured distribution coefficients had been surveyed in more detail.

D.F. Weill, R. Hon and A. Navrotsky next illustrate the progress made, despite considerable difficulties, in the prediction of crystal-liquid phase equilibria from thermodynamic data. In this context, it is worthy of note that experimental determination has a more important future than some would have predicted five years ago. Progress in determination of the important physical parameters of magmas is covered by I. Kushiro. He emphasizes the importance of the transition from four-fold to six-fold coordination of aluminium in controlling increase of density, decrease of viscosity and change of liquid structure in the 10–20 kb range. (The mind leaps to the possible effects of the same transition in the coordination of silicon, which should produce yet more dramatic changes of such properties at pressures encountered within the lower part of the upper mantle — it is a pity that Kushiro does not speculate on this.) He does, however, discuss the overall implications of the changes of viscosity and density for rates of magma movement, changes in volatile solubility and the efficiency of magma extraction, crystal-

liquid fractionation and diffusive processes.

S.R. Hart and C.J. Allegre review the field of trace element and isotope geochemistry, before going on to provide an excellent summary of the "traditional" (or pre-1980) theory of the petrogenesis of mafic and granitic rocks. The more complicated but entirely plausible trace element models (touched on briefly in this chapter), combined with new understanding of the physics of magma mixing, have transformed thinking on the subject; fractionating magma chambers fed by uniform parent liquids can erupt lava suites which must (traditionally) be interpreted as partial melting products of heterogeneous mantle sources. The rehabilitation of assimilation and contamination is at hand, and increasing evidence for diffusive differentiation of trace elements (and isotopes?) in magma chambers may shortly overwhelm all other considerations. These are stirring times!

In his critical review of problems in heat flow determination and interpretation, E.R. Oxburgh concentrates on a survey of partial melting in upward convecting systems. Somewhere in this book it would have been appropriate to refer to the vexed question of density constraints on mantle plumes and to the inverse possibility that partial melting at the base of the mantle precedes and drives the convective motions. An unconsidered possibility is that partial melts may become denser than olivine (but not denser than a bulk residue containing silica-rich garnet rather than pyroxene) near the base of the upper mantle, hence allowing upward motion of low percentage partial melts, downward movement of high percentage melts and complex redistribution of heat-producing elements.

Whereas the first five chapters are models of clarity, H.R. Shaw's contribution is distinctly tough going. In almost mystic prose he explores possible relationships between stress fields, rates and volumes of magma flow, and seismicity. Nevertheless, I shall be reading and re-reading this section in order to understand its implications for the (rather neglected) situations where major magma chambers intervene between source and vent, and changes in magma composition

and density may become key factors in controlling magma transport. In the following contribution, F.J. Spera covers some of the same ground as Shaw but extends the discussion to many other aspects of the segregation, ascent and eruption of magmas. Major evolution of liquid composition probably occurs between source and point of eruption in most volcanic systems, and will complicate further the relationships discussed here.

In a thoughtful and stimulating contribution, T.N. Irvine documents the recent revolution in ideas concerning chemical and physical processes in slowly cooled magma chambers. This will be essential reading for anyone interested in consolidation of magmas. Irvine covers diagenesis of crystal accumulations but unfortunately only touches on fluid dynamics; it is in this latter field that some of the most spectacular progress has been made since the book went to press.

A.W. Hoffman deals with one- and two-way diffusion in magmas, making particular reference to determinative methods rather than specific applications. One of the most important questions which is discussed in detail by Hoffman concerns the possible influence of diffusion through the boundaries between individually well-mixed convecting layers in a cooling magma chamber. While crystal-liquid equilibria apply a constraint on the lowest layer — at least if the chamber is solidifying by bottom-crystallization — it does not follow that the higher layers of the magma from which lava flows are likely to be derived will be in major- or trace-element equilibrium with the "cumulate" assemblage, or even in isotopic equilibrium with it.

E. Dowty deals with the basic theory of crystal nucleation and growth. He reviews a range of factors which may affect the morphology and chemistry of growing (or dissolving) crystals, and thus the development of grain size distributions and textures of igneous rocks, stressing once again the value of experimental work. G. Lofgren continues the story of the experimental study of crystal growth and texture development, especially with regard to lunar and terrestrial basic magmas. This final contribution provides a timely review of recent activities in the field.

Like its companion volume, this book appears at the moment when igneous petrology is making its most rapid advances since Bowen's day. Consequently it, too, suffers from omissions which will prevent it from standing as a milestone on the long road towards our understanding of magmatic processes; rather, it should be appreciated as a speedometer. Nevertheless, I found the book stimulating reading and recommend it to all serious students of igneous petrology. □

M.J. O'Hara is Professor and Head of the Geology Department, University College of Wales, Aberystwyth.

BOOKS RECEIVED

Mathematics

- BERGER, J. O. *Statistical Decision Theory. Foundations, Concepts and Methods*. Pp.425. ISBN 3-540-90471-9. (Springer-Verlag: 1980.) DM 45, \$26.60.
- HELLEMAN, R. H. G. (ed.). *Nonlinear Dynamics. Annals of the New York Academy of Sciences*, Vol.347. Pp.505. Hbk ISBN 0-89766-103-6; pbk ISBN 0-89766-104-4. (The New York Academy of Sciences, New York: 1980.) Hbk \$98; pbk np.
- NICKEL, K. L. E. (ed.). *Interval Mathematics 1980. Proceedings of an International Symposium held at the Institut für Angewandte Mathematik Universität Freiburg i. Br., Germany, May 1980*. Pp.554. ISBN 0-12-518850-1. (Academic: 1980.) \$29.50.
- PLUMPTON, C. and MACILWAINE, P. S. W. *New Tertiary Mathematics. Vol.1; Part 1, Pure Mathematics: The Core*. Pp.401. Hbk ISBN 0-08-025031-9; flexi ISBN 0-08-021643. (Pergamon: 1981.) Hbk £12.50; flexi £5.
- PLUMPTON, C. and MACILWAINE, P. S. W. *New Tertiary Mathematics. Vol.1; Part 2, Basic Applied Mathematics*. Pp.229. Hbk ISBN 0-06-025035-1; flexi ISBN 0-08-021645-5. (Pergamon: 1981.) Hbk £12.50; flexi £5.
- PLUMPTON, C. and MACILWAINE, P. S. W. *New Tertiary Mathematics. Vol.2; Part 1, Further Pure Mathematics*. Pp.804. Hbk ISBN 0-08-025033-5; flexi ISBN 0-08-021644-7. (Pergamon: 1981.) Hbk £17.40; flexi £5.
- ROGERS, C. A. *et al.* *Analytic Sets. Developed from Lectures given at the London Mathematical Society Instructional Conference on Analytic Sets, University College London, July 1978*. Pp.495. ISBN 0-12-593150-6. (Academic: 1980.) £48, \$110.50.
- ZACKS, S. *Parametric Statistical Inference. Basic Theory and Modern Approaches. International Series in Nonlinear Mathematics: Theory, Methods and Applications*, Vol.4. Pp.387. (Pergamon: 1981.) £20, \$48.

Physics

- BORN, M. and WOLF, E. *Principle of Optics. Electromagnetic Theory of Propagation, Interference and Diffraction of Light*. 6th Edn. Pp.808. Hbk ISBN 0-08-026482-4; flexi ISBN 0-08-026481-6. (Pergamon: 1980.) Hbk np; flexi £12.
- FERRARI, E. and VIOLINI, G. (eds). *Low and Intermediate Energy Kaon-Nucleon Physics. Proceedings of the Workshop, Institute of Physics of the University of Rome, March 1980*. Pp.428. ISBN 90-277-1183-6. (Reidel: 1981.) Dfl.100.
- GRIFFITHS, D. J. *Introduction to Electrodynamics*. Pp.479. ISBN 0-13-481374-4. (Prentice-Hall International: 1981.) £15.55.
- MAHAN, G. D. *Many-Particle Physics. Physics of Solids and Liquids*. Pp.1003. ISBN 0-306-40411-7. (Plenum: 1981.) \$85.
- MULLER, K. A. and THOMAS, H. (eds). *Structural Phase Transitions 1. Topics in Current Physics*, Vol.23. Pp.190. ISBN 3-540-10329-5. (Springer-Verlag: 1981.) DM 50, \$29.50.
- PERSONICK, S. D. *Optical Fiber Transmission Systems. Applications of Communications Theory Series*. Pp.179. ISBN 0-306-40580-6. (Plenum: 1981.) Np.
- RAMOND, P. *Field Theory. A Modern Primer. Frontiers in Physics*, No.51. Pp.397. Hbk ISBN 0-8053-7892-8; pbk ISBN 0-8053-7893-6. (Benjamin/Cummings, Menlo Park, California: 1981.) Hbk \$26.50; pbk \$14.50.
- RAPP, D. *Solar Energy*. Pp.516. ISBN 0-13-822213-4. (Prentice-Hall: 1981.) \$32.
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- SOBELMAN, I. I., VAINSHTEIN, L. A. and YUKOV, E. A. *Excitation of Atoms and Broadening of Spectral Lines*. Pp.328. ISBN 3-540-09890-9. (Springer: 1981.) DM 75, \$44.30.

Chemistry

- BRADY, J. E. and HOLM, J. R. *Study Guide. Fundamentals of Chemistry*. Pp.396. Flexi ISBN 0-471-05817-3. (Wiley: 1981.) Np.
- DENBIGH, K. *The Principles of Chemical Equilibrium. With Applications in Chemistry and Chemical Engineering*. 4th Edn. Pp.494. Hbk ISBN 0-521-23682-7; pbk ISBN 0-521-28150-4. (Cambridge University Press: 1981.) Hbk £22; pbk £8.95.
- ENGLMAN, R. *et al.* *Bonding Problems. Structure and Bonding*, Vol.43. Pp.220. ISBN 3-540-10407-0. (Springer-Verlag: 1981.) DM 98, \$57.90.
- INMAN, D. and LOVERING, D. G. (eds). *Ionic Liquids*. Pp.450. ISBN 0-306-40412-5. (Plenum: 1981.) \$49.50.
- ISAACS, N. S. *Liquid Phase High Pressure Chemistry*. Pp.414. ISBN 0-471-27849-1. (Wiley: 1981.) Np.
- KATRITZKY, A. R. and BOULTON, A. J. (eds). *Advances in Heterocyclic Chemistry*, Vol.27. Pp.331. ISBN 0-12-020627-7. (Academic: 1981.) \$49.50.
- LOWDIN, P.-O. *Advances in Quantum Chemistry*, Vol.12. Pp.325. ISBN 0-12-034812-8. (Academic: 1980.) \$48.
- MCGUIRE, S. Y. *Study Guide for Chemistry. An Introduction to General, Organic and Biological Chemistry*. Pp.307. Pbk ISBN 0-7167-1314-4. (W. H. Freeman: 1981.) \$7.95.
- MEITES, L. *An Introduction to Chemical Equilibrium and Kinetics. Pergamon Series in Analytical chemistry*, Vol.1. Pp.549. Hbk ISBN 0-08-023802-5; flexi ISBN 0-08-023803-3. (Pergamon: 1981.) Hbk £31, \$75; flexi £8.95, \$19.95.

- MIDDLEDITCH, B. S. MISSLER, S. R. and HINES, H. B. *Mass Spectrometry of Priority Pollutants*. Pp.308. ISBN 0-306-40505-9. (Plenum: 1981.) Np.
- PAMPLIN, B. R. (ed.). *Progress in Crystal Growth and Characterization*, Vol.2. Pp.414. ISBN 0-08-026040-3. (Pergamon: 1981.) £49, \$112.50.
- PATAKI, L. and ZAPP, E. *Basic Analytical Chemistry. Pergamon Series in Analytical Chemistry*, Vol.2. Pp.475. ISBN 0-08-023850-5. (Pergamon: 1980.) £15.50, \$35.
- QUIN, L. D. *The Heterocyclic Chemistry of Phosphorus: Systems Based on the Phosphorus-Carbon Bond*. Pp.434. ISBN 0-471-06461-0. (Wiley: 1981.) £27.05, \$59.50.
- RICHTERICH, R. and COLOMBO, J. P. *Clinical Chemistry. Theory, Practice, and Interpretation*. Pp.766. ISBN 0-471-278092. (Wiley: 1981.) £31.50, \$94.50.
- SMITH, R. and JAMES, G. V. *The Sampling of Bulk Materials*. Pp.191. ISBN 0-85186-810-X. (The Royal Society of Chemistry, London: 1981.) £16.50.
- STEINFELD, J. I. (ed.). *Laser-Induced Chemical Processes*. Pp.273. ISBN 0-306-40587-3. (Plenum: 1981.) Np.
- WIDOM, J. M. and EDELSTEIN, S. J. *Chemistry. An Introduction to General, Organic, and Biological Chemistry*. Pp.740. ISBN 0-7167-1224-5. (W. H. Freeman: 1981.) \$22.95.
- ZAHRADNIK, R. and POLAK, R. *Elements of Quantum Chemistry*. Pp.462. ISBN 0-306-31093-7. (Plenum: 1980.) \$29.50.

Technology

- CLARK, A. F. and REED, R. P. (ed). *Advances in Cryogenic Engineering Materials Vol.26. Proceedings of the 3rd International Conference, the 1979 Cryogenic Engineering Conference, held at the University of Madison, Wisconsin, August 1979*. Pp.703. ISBN 0-306-40531-8. (Plenum: 1980.) \$59.
- DE SA, A. *Principles of Electronic Instrumentation*. Pp.280. ISBN 0-7131-2799-6. (Edward Arnold, London: 1981.) \$9.50.
- POLLOCK, J. R. A. (ed.). *Brewing Science*, Vol.2. Pp.628. ISBN 0-12-561002-5. (Academic: 1981.) £41, \$98.50.

Earth Sciences

- DAVIES, P. A. and RUNCORN, S. K. (eds). *Mechanisms of Continental Drift and Plate Tectonics. Preceedings of a NATO Advanced Study Institute held at the University of Newcastle upon Tyne, March-April, 1979*. Pp.362. ISBN 0-12-206160-8. (Academic: 1981.) £30 \$72.
- ELDER, J. *Geothermal Systems*. Pp.508. ISBN 0-12-236450-3. (Academic: 1981.) £20.60, \$49.50.
- GRIFFITHS, D. H. and KING, R. F. *Applied Geophysics for Geologists and Engineers: The Elements of Geophysical Prospecting. (A 2nd Edn. of Applied Geophysics for Engineers and Geologists)*. Pp.230. Hbk ISBN 0-08-022071-1; flexi ISBN 0-08-02272-X. (Pergamon: 1981.) Hbk £11, \$25; flexi £5.30, \$12.
- PANAYIOTOU, A. (ed.). *Ophiolites. Proceedings International Ophiolite Symposium, Cyprus 1979*. Pp.781. (Republic of Cyprus Ministry of Agriculture and National Resources Geological Survey Department: 1980.) £15.
- TUCKER, M. E. *Sedimentary Petrology. An Introduction. Geoscience Texts*, Vol.3. Pp.252. Flexi ISBN 0-632-00072-0. (Blackwell Scientific: 1981.) £8.50.

Biological Sciences

- ADLERCREUTZ, H. *et al.* (eds). *Endocrinological Cancer Ovarian Function and Disease. Research on Steroids*, Vol. IX. Pp. 400. ISBN 90-219-0444-6. (Excerpta Medica, Amsterdam: 1981.) \$78, Dfl.160.
- ANGELINI, C., DANIELI, G. A. and FONTANARI, D. (eds). *Muscular Dystrophy Research: Advances and New Trends. Proceedings of an International Symposium, Venice, Italy, April 1980*. Pp.332. ISBN 90-219-0453-5. (Excerpta Medica, Amsterdam: 1980.) \$68.25, Dfl.140.
- APPLEWHITE, P. B. *Molecular Gods. How Molecules Determine our Behaviour*. Pp.262. ISBN 0-13-599530-2. (Prentice-Hall: 1981.) \$10.95.
- BACH, F. H. and GOOD, R. A. (eds). *Clinical Immunobiology*, Vol.4. Pp.198. ISBN 0-12-070004-2. (Academic: 1980.) \$19.50.
- BHASKARAN, G., FRIEDMAN, S. and RODRIGUEZ, J. G. (eds). *Current Topics in Insect Endocrinology and Nutrition. Proceedings of the Symposium honouring Gottfried S. Fraenkel, the Entomological Society of America, November 1979, Denver, Colorado*. Pp.362. ISBN 0-306-40621-7. (Plenum: 1981.) \$29.50.
- BOLAND, D. J. *et al.* *Eucalyptus Seed*. Pp.191. ISBN 0-643-02586-3. (Division of Forest Research, CSIRO, Canberra, Australia: 1980.) \$18.
- GILLESPIE, J. H. and TIMONEY, J. F. *Hagan and Bruner's Infectious diseases of Domestic Animals. With Reference to Etiology, Pathogenicity, Immunity, Epidemiology, Diagnosis, and Biologic Therapy*. 7th Edn. Pp.851. ISBN 0-8014-1333-8. (Cornell University Press: 1981.) \$39.50.
- GLOVER, D. M. *Genetic Engineering Cloning DNA*. Pp.78. Flexi ISBN 0-412-16170-2. (Chapman & Hall: 1980) £2.45.
- GOLDSPINK, D. F. (ed.) *Development and Specialization of Skeletal Muscle*. Pp.155. Hbk ISBN 0-521-23317-8; pbk ISBN 0-521-29907-1. (Cambridge University Press: 1981.) Hbk £18, pbk £8.95.
- GOODERS, J. *The Bird Seeker's Guide*. Pp.208. Pbk ISBN 0-233-97297-8; pbk ISBN 0-233-97380-X. (André Deutsch: 1981.) Hbk £6.95; Np.
- HARRIS, E. and HARRIS, J. *The Guinness Book of Trees. Britain's Natural Heritage*. Pp.160. ISBN 0-85112-303-1. (Guinness Superlatives, Enfield, Middlesex: 1981.) £4.50.

ANNOUNCEMENTS

Awards

The 1981 recipients of the Harvey Prize of the Technion — Israel Institute of Technology are **Prof. Sir James Lighthill** (Provost of University College, London, UK) receives the Harvey Prize in Science and Technology; **Prof. Hans W. Kosterlitz** (Director of the Unit on Addictive Drugs, University of Aberdeen, UK) receives the Harvey Prize in Human Health.

The winners of the 1981 General Motors Cancer Research Foundation Awards are **Dr Wallace Prescott Rowe** (Chief of the Laboratory of Viral Diseases at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland) for his contributions to virology; **Dr E. Donnell Thomas** (Director of Medical Oncology at the Fred Hutchinson Cancer Research Center in Seattle, Washington) for developing bone marrow transplantation as a successful treatment for acute leukemia and aplastic anemia; **Dr Cesar Milstein** (Medical Research Council Laboratory of Molecular Biology in Cambridge, UK) for developing hybridoma technology; **Dr Takashi Sugimura**, (Director of Tokyo's National Cancer Center Research Institute) for identifying possible cancer causing substances in our food.

The Low Temperature Group of the Institute of Physics has awarded the Simon Memorial Prize to **Prof. A. J. Leggett** (University of Sussex), for work on the theory of superfluid ^3He .

The Trustees of the Lady Tata Memorial Trust have made international awards for research in leukaemia to: **Dr B. Azzarone** (Italy), **Dr A.D. Crockard** (UK), **Dr S. Venuta** (Italy) — *renewed awards*. **Dr H.D. Abrams** (USA), **H.J. Allen** (UK) — *new awards*.

The International Meteorological Organization (IMO) Prize, for outstanding work in meteorology or operational hydrology and international cooperation has been awarded to **Prof. Bert Bolin** (Director of the International Meteorological Institute, Stockholm).

The Beit Memorial Fellowships for medical research 1981 have been awarded to **David Leonard Bentley** (MRC Laboratory for Molecular Biology, Cambridge, UK); **Stephen Eric Kearsey** (MRC Laboratory for Molecular Biology, Cambridge, UK); **David Ian Vaney** (The Physiological Laboratory, Cambridge University, UK); **John Vincent Priestley** (Dept of Pharmacology, Oxford University, UK); **Peter John Richardson** (Dept of Clinical Biochemistry, University of Cambridge Medical School, New Addenbrooke's Hospital, UK).

Meetings

21-22 August, **Excitation-Contraction Coupling in Skeletal, Cardiac and Smooth Muscle**, Banff (Dr George B. Frank, Dept of Pharmacology, The University of Alberta, Edmonton, Alberta, Canada T6G H77).

25-27 August, **Life Sciences in the Service of Alaska**, Fairbanks (Conferences and Institutes, The University of Alaska, Fairbanks, Alaska 99701, USA).

2-4 September, **Modern Aspects of Phase Equilibria**, Teesside (Mrs M. Findley, Dept of Chemical Engineering, Teesside Polytechnic, Borough Rd, Middlesbrough, Cleveland, UK).

1-7 September, **Fundamentals of Microprocessors**, London (Course Registrar, Bleasdale Computer Systems Ltd, Francis St, London SW1, UK).

6-12 September, **32nd International Astronautical Congress**, Rome (Prof. Luigi G. Napolitano, International Astronautical Federation, 250 Rue Saint-Jacques, 75005 Paris, France).

7 September, **Water Quality**, London (R. Gardam, Thames Water Authority, New River Head, Rosebury Avenue, London EC1, UK).

7-8 September, **Chemical Aspects of Changes in Food on Storage**, Norwich (Dr R. Fenwick, Society of Chemical Industry, Food Group, 14 Belgrave Square, London SW1, UK).

7-10 September, **EMAG '81**, Cambridge (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

7-11 September, **Chemistry of Carbocations**, Bangor (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).

7-11 September, **An Introduction to Hydrometallurgy**, Hatfield (D.B. Firth, Institution of Chemical Engineers, 12 Gayfere St, London SW1, UK).

10-11 September, **National Conference on Fitness and Aging**, Washington DC (U.A. Wheaton, President's Council, 400-6th Street S.W. Room 3030, Washington DC 20201, USA).

16-18 September, **Chemical Engineering Education**, London (C.J. Bullock, Chemical Engineering Dept, Teesside Polytechnic, Middlesbrough, Cleveland, UK).

17-18 September, **Regulation of Energy Balance**, Edinburgh (Dr J.A. Milne, Hill Farming Research Organisation, Penicuik, Midlothian, UK).

19-21 September, **Teilhard and Metamorphosis**, Arizona, (Arcosanti Events, 6433 Doubletree Rd, Scottsdale, Arizona 85253, USA).

22 September, **Autonomic Nerves of the Gut**, London (Dr M. Daly, Glaxo Group Research Ltd, Ware, Herts, UK).

22-24 September, **Royal Society of Chemistry Autumn Meeting**, Leeds (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).

23 September, **Information Meeting for Nature Protection Societies from the European Community**, Brussels (Ms J. White, EEB, 31 rue Vautier, B-1040 Bruxelles, Belgium).

23-25 September, **Wave and Tidal Power**, Cambridge (Conference Organiser, Wave and Tidal Energy, BHRA Fluid Engineering, Cranfield, Bedford, UK).

24-25 September, **Platinum Group and Gold Mineralization, Ultramafic Rocks, and Zeolites**, Manchester (Dr C.M.B. Henderson, Dept of Geology, The University, Manchester, UK).

24-25 September, **The World Conservation Strategy and the European Communities**, Brussels (Ms J. White, EEB, 31 rue Vautier, B-1040 Bruxelles, Belgium).

25-28 September, **Eocene and Oligocene of the Isle of Wight**, Sandown (Dr A.N. Insole, Museum of Isle of Wight Geology, Sandown Library, High St, Sandown, Isle of Wight, UK).

28 September — 2 October, **Annual Meeting European Society of Nuclear Methods in Agriculture**, Aberdeen (Mr A.H. Knight, Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, UK).

28 September — 2 October, **Dynamics of Turbid Coastal Environments**, Nova Scotia (D.C. Gordon, Marine Ecology Laboratory, Bedford Institute of Oceanography, PO Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2).

28 September-17 October, **New Approaches to Plant Genetics and Breeding**, Peking (Dr O.L. Gamborg, International Plant Research Institute, 887 Industrial Rd, San Carlos, California 94070, USA).

29 September, **Gas Cyclones**, London (Mrs E.H. Edwards, Institution of Chemical Engineers, 12 Gayfere St, London SW1, UK).

30 September — 2 October, **Deburring '81**, New Orleans (J. Slaughter, Technical Activities Dept, Society of Manufacturing Engineers, 1 SME Drive, PO Box 930, Dearborn, Michigan 48128, USA).

2-3 October, **21st Anniversary Meeting of Radiologists**, Dublin (Miss A. Daly, Faculty of Radiologists, Royal College of Surgeons in Ireland, 123 St Stephens Green, Dublin 2, Ireland).

5-7 October, **Wind Energy Conference and Workshop**, Washington (Conferences Group, SERI, 1617 Cole Boulevard, Golden, Colorado 80401, USA).

5-8 October, **Pollution Control**, Brighton (NSCA, 136 North St, Brighton, Sussex, UK).